The performance of the Zebrafish Embryo Test (ZET) in predicting the presence and absence of malformations in the studies of prenatal development toxicity in rats and rabbits (OECD TG 414 and equivalents). A systematic review.

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Review question

To determine the performance of the Zebrafish Embryo Test in predicting the presence or absence of malformations in rats and rabbits* following chemical exposure in the OECD's Test Guideline 414 or similar tests.

Context and rationale

Fuelled by the Thalidomide disaster around 1960, the assessment of the hazard of chemicals to adversely interfere with the pre-natal human development was implemented into many regulations worldwide. In 1981 the OECD adopted its Test Guideline 414, which was amended most recently in 2001, thus providing an internationally accepted standard procedure to assess this hazard using animal models.

Substantial, but poorly understood, species differences have led in some regulations to the requirement to conduct the OECD414 in two species. As this traditional test is cost and time intensive and uses a considerable number of animals, there is broad interest in less expensive and sufficiently predictive tests that allow a higher throughput.

The modelling of pre-natal development remains a challenge, not least because of the complexity of the process and possible perturbation thereof, which is still an area where detailed understanding is lacking. While a number of in vitro and ex vivo tests for developmental toxicity have been developed, no one test or combination of such tests can yet substitute fully for the guideline tests. However, the zebrafish embryo test (ZET), which offers practical (cost, throughput) and ethical (e.g., no maternal killing) advantages, is considered promising. It has been and still is intensively studied, so that a considerable amount of data is available.

Therefore, the present review is designed to assess the predictive performance of ZET in comparison to mammalian pre-natal developmental toxicity, allowing an objective evaluation of its promise.

Searches

Sources searched:

- BIOSIS
- Embase
- PubMed
- Toxline

Language restrictions: Studies not reported in English will be excluded.

Mammalian developmental toxicity tests: Studies published before June 22, 2016, i.e. the date of the ZET search.

Search strategy

https://www.crd.york.ac.uk/PROSPEROFILES/96120_STRATEGY_20180509.pdf

Types of study to be included

Inclusion criteria:
Experimental animal studies with controlled exposure and a separate control group.

Exclusion criteria:
Experimental animal studies without controlled exposure or without a separate control group are excluded. Studies on humans (any type) are outside the scope.

Human disease modelled

Hazard of chemicals to adversely interfere with the pre-natal human development, leading especially to malformations.

Animals/population

Inclusion criteria:
Zebrafish embryo test:
- Studies conducted on zebrafish embryos
- Studies conducted on wild-type Zebrafish embryos in which the species' strain is reported (e.g. AB, AB/TU, SAT, TU, SJD, SJA, WIK, TL)
Mammalian developmental toxicity tests:
- Studies conducted on rats or rabbits
- Studies conducted on wild-type rats or rabbits in which the species' strain is reported

Exclusion criteria:
Zebrafish embryo test:
- Studies conducted on species other than Zebrafish
- Studies conducted on mutant Zebrafish strains (e.g. transgenic strains)
Mammalian developmental toxicity tests:
- Studies conducted on species other than rats and rabbits
- Studies performed in mutant rats and rabbits (e.g. transgenic animals)
- Studies conducted according to the Organization for Economic Cooperation and Development (OECD) Test Guideline 414 (Prenatal Developmental Toxicity Study) and similar tests (refered to as OECD TG 414)

Intervention(s), exposure(s)

Inclusion criteria:
Zebrafish embryo test:
- Studies conducted on individual chemicals, in which the test substance(s) identity is given (i.e. by reporting a CAS number).
- Studies reporting on at least three chemical concentrations excluding the control
- Studies starting exposure 0 to 6 hours post fertilization
- Studies with outcome assessment of 48 to 120 hours post fertilization (hpf)
Mammalian developmental toxicity tests:
- Studies conducted on the same individual chemicals as those from included zebrafish studies, with the test substance(s) identity specified (i.e. by reporting the CAS number or the chemical's common name, from which the CAS no. was identified)
- Studies, in which doses were administered orally via gavage or in food
- Studies reporting on at least three chemical doses
- Start of exposure not earlier than gestational day 5
- End of exposure not earlier than gestational day 15 in rats or day 19 in rabbits
- Examination of fetuses pre-birth not earlier than gestational day 19 in rats or day 26 in rabbits

Exclusion criteria:
Zebrafish embryo test:

- Studies conducted on chemicals that are not clearly identified (i.e. chemicals with no CAS No. reported, mixtures, plant extracts, nanochemicals, ecological chemicals in water sources)
- Studies reporting on less than three chemical concentrations
- Studies starting exposure after 6 hours post fertilization
- Studies with observations outside the range of 48 to 120 hpf

Mammalian developmental toxicity tests:
- Studies conducted on test chemicals not also studied in zebrafish
- Studies, in which chemicals were administered in a way other than gavage or in food (e.g. intraperitoneal, inhalation, intravenous)
- Studies reporting on less than three chemical concentrations
- Start of exposure earlier than gestational day 5
- End of exposure earlier than gestational day 15 in rats or day 19 in rabbits
- Examination of fetuses pre-birth earlier than gestational day 19 in rats or day 26 in rabbits

Comparator(s)/control

Inclusion criteria:
Inclusion criteria for the comparator test, i.e. mammalian developmental toxicity test, are described above under 19. and 20. In addition, a negative and/or vehicle control group should have been included in individual studies.

Exclusion criteria:
Exlusion criteria for the comparator test, i.e. mammalian developmental toxicity test, are described above under 19. and 20. In addition, individual studies without a negative and/or vehicle control group are excluded.

Other selection criteria or limitations applied

Inclusion of mammalian developmental toxicity tests:
Studies published before June 22, 2016, i.e. the date of the ZET search. (Studies published later are excluded).

Outcome measure(s)

Inclusion criteria:
Zebrafish embryo test:
- Studies that look at outcome measures that represent all of the following 3 types of developmental toxicity endpoints: mortality, general and specific embryotoxicity (Annex 2)
- Studies with at least 10 eggs per concentration

Mammalian developmental toxicity tests:
- Studies that look at outcome measures that represent all of the following 4 types of developmental toxicity endpoints: growth retardation, external, soft tissue and skeletal abnormalities (Annex 3).

- Studies with at least 16 animals reported per group

Exclusion criteria:
Zebrafish embryo test:
- Studies that did not include outcome measures for all of the following 3 types of developmental toxicity endpoints: mortality, general and specific embryotoxicity (Annex 2) (e.g. studies that only report hatching rate or embryo mortality).
- Studies with less than 10 eggs per concentration

Mammalian developmental toxicity tests:
- Studies that did not include outcome measures for all of the following 4 types of developmental toxicity endpoints: growth retardation, external, soft tissue and skeletal abnormalities (e.g. studies that only report embryo mortality or maternal toxicity).
- Studies with less than 16 animals per group

Study selection and data extraction

Procedure for study selection
Zebrafish embryo test:

The resulting citations will be screened based on the title and abstract by two individuals for eligibility based on the zebrafish inclusion and exclusion criteria. For each citation deemed potentially eligible the full article will be retrieved to assess the remaining inclusion and exclusion criteria.

Mammalian developmental toxicity tests:
Subsequently, the chemicals identified in qualified ZET studies will be used to retrieve eligible studies in rats and rabbits through the identified electronic search strategy. Citations deemed eligible will have the full article retrieved. A second screening of full text of all retrieved studies will be performed to determine final eligibility. If no eligible studies are retrieved, then the chemical and all previously selected zebrafish studies will be excluded.
Number of reviewers:
(a) Two individual researchers in all selection phases.
(b) The original study will be consulted to make a decision when there is discrepancy between the results returned by the two individual researchers. Such a decision should be made after discussion between the two individual researchers and referring to the selection criteria. If consensus is not reached through the discussions, the final decision will be taken by the authors.

Prioritise the exclusion criteria
Selection phase for Zebrafish embryo test studies:
- Tiab: 1. Developmental toxicity outcomes (all types); 2. Species and non-mutant; 3. Exposure to single chemical; 4. Non-English; 5. Original data; 6. Minimum number of eggs per concentration; 7. Minimum number of concentrations; 8. Onset of exposure; 9. Time point of outcome assessment
- Full text: 1. Developmental toxicity outcomes (all types); 2. Species and non-mutant; 3. Exposure to single chemical; 4. Non-English; 5. Original data; 6. Minimum number of eggs per concentration; 7. Minimum number of concentrations; 8. Onset of exposure; 9. Time point of outcome assessment

Selection phase for mammalian developmental toxicity studies:
- Tiab: 1. Developmental toxicity outcomes (all types); 2. Species and non-mutant; 3. Exposure to single chemical; 4. Non-English; 5. Original data; 6. Administration route; 7. Minimum number of animals per group; 8. Minimum number of concentrations; 9. Onset of exposure; 10. Time point of outcome assessment
- Full text: 1. Developmental toxicity outcomes (all types); 2. Species and non-mutant; 3. Exposure to single chemical; 4. Non-English; 5. Original data; 6. Administration route; 7. Minimum number of animals per group; 8. Minimum number of concentrations; 9. Onset of exposure; 10. Time point of outcome assessment

Methods for data extraction
Data will be extracted from all the text, tables and graphs and their legends.
A standardized data extraction form will be developed. Data will be extracted from all eligible studies by one reviewer and random quality control will be performed by a project manager. If there are any issues with extraction, the authors will be consulted in making the final decision.

Data to be extracted: study design
not applicable

Data to be extracted: animal model
- Species and strain studied (zebrafish, rats, rabbits)

- Name of chemical (with CAS number)

Data to be extracted: intervention of interest
- Doses or concentrations used
- Exposure route (oral via gavage or diet)
- Time and duration of exposure
- Type of control groups (negative/vehicle and/or positive controls)
- Number of eggs or animals per concentration

Data to be extracted: primary outcome(s)
There will be three classifications of studies with respect to developmental toxicity:
- positive: chemical studied is a developmental toxicant
- negative: chemical studied is not a developmental toxicant
- inconclusive: there are insufficient data (reported) in the study to classify the chemical as positive or negative

The criteria for classifying a study as positive or negative differ between the two test methods that are compared.

Zebrafish embryo test:

A study will be classified as positive for embryotoxicity for a specific chemical if it reports:
a) the occurence of any observation of the parameters of general or specific embryotoxicity identified in Annex 2 at any concentration and any time point (between 48 and 120h)

A study will be classified as negative for embryotoxicity for a specific chemical if it reports :
a) either no observation of any of the parameters of general or specific embryotoxicity identified in Annex 2 and the highest concentration studied is at least 1,000 uM, has reached the solubility limit or is otherwise considered to be sufficiently high. (Note that for this purpose, the dose range and justification of Cmax will be extracted.)
or
b) mortality at all concentrations and time points (between 48 and 120h) without any specific embryotoxicity

A study will be classified as inconclusive for embryotoxicity for a specific chemical if does not report observations of developmental toxicity, but:
a) did not look at all of the parameters of general or specific embryotoxicity identified in Annex 2, or
b) the highest concentration is considered not to be sufficiently high.

Mammalian developmental toxicity tests:

A study will be classified as positive for embryotoxicity for a specific chemical if it reports:
a) either the occurence of any observation classified in Annex 3 as malformation at any dose or
a statistically significant higher incidence at any dose of any observation classified in Annex 3 as a variation, whereby the dose at which the malformation or higher incidence of a variation occured was equal to or lower than the dose causing maternal toxicity. (Note that for this purpose, both the maternal L/NO(A)EL and fetal LOEL will be extracted.)

A study will be classified as negative for embryotoxicity for a specific chemical if it reports observations of all categories of Annex 3 and

a) either finds no malformation or no statistically significant difference in incidence of variation at any of the doses studied, whereas the highest dose level included was toxic at maternal level or at least 1000 mg/kg bodyweight (bw). (Note that for this purpose, both the maternal L/NO(A)EL and fetal NO(A)EL will be extracted) or

b) the dose at which the malformation or higher incidence of a variation occurs is higher than the dose that caused maternal toxicity

A study will be classified as inconclusive for embryotoxicity for a specific chemical if reports no malformation or statistically significant difference in incidence of a variation, but:
a) did not look at all categories of Annex 3, or
b) the highest dose level included was not maternally toxic or lower than 1000 mg/kg bw.

Data to be extracted: secondary outcome(s)
none

Data to be extracted: other
Article identifier (e.g., author and year)

Risk of bias and/or quality assessment

Strategy for data synthesis

Planned approach
A quantitative synthesis is planned as described below. However, no meta-analysis is planned.

Effect measure
From tabulated results including the chemical identity and the dichotomized results of the index and the comparator test (excluding studies that are inconclusive), a 2x2 contingency table will be constructed for each comparison (zebrafish vs rat, zebrafish vs rabbit, zebrafish vs combined rat and rabbit (positive, if at least one species positive)), from which the following percentages of concordance and discordance will be calculated:

- n22: number of chemicals which both the ZET and the OECD TG 414 test(s) identified as ‘positive’
- n11: number of chemicals which both the ZET and the OECD TG 414 test(s) identified as ‘negative’
- n12: number of chemicals that the ZET identified as ‘negative’ and the OECD TG 414 test(s) as ‘positive’.
- n21: number of chemicals that ZET identified as ‘positive’ and the OECD TG 414 test(s) as ‘negative’.

Confidence intervals will be calculated for n11/n.1, which may also be referered to as specificity, and n22/n.2, which may also be referred to as sensitivity, to reflect the precision of these estimates.

Multiple ZET studies of a given substance with discordant results will be evaluated for experimental reasons potentially explaining the discordance, such as differences in exposure or study duration. When unexplainable discordant results are found for more than 5% of the total of included substances, this may substantial impact the results of the comparisons. In that case distributions of n11/n.1 and n22/n.2 will be calculated by using bootstrapping (using the multiple study results), following the approach e.g. presented by Alépée et al. (Toxicol in Vitro, 2015, 30(1 Pt B):373-382). If there are less than 5% substances with unexplainable discordant zebrafish studies, the substances zebrafish results will be determined by the mode of the results of the repeat studies.
Discordant repeat studies in rat or rabbits will lead to the exclusion of the respective substance if the ratio ‘negative/positive’ of the study results ranges between 1/3 (=25%/75%) and 3 (75%/25%). When the ratio is smaller than 1/3 or larger than 3, the more frequent results will be used.

Effect models
not applicable

Heterogeneity
not applicable

Other
not applicable

Analysis of subgroups or subsets

Subgroup analyses
No subgroup analyses will be performed. Study characteristics that may be of interest for future analysis include protocol variants for the ZF studies (e.g., dechorionated versus chorionated embryos) and aspects of study quality.

Sensitivity
not applicable

Publication bias
None planned

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Conflicts of interest
Burkhard Flick works for BASF (chemical industry)

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(there is not an English language summary)

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United States of America

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https://www.crd.york.ac.uk/PROSPEROFILES/96120_PROTOCOL_20180509.pdf

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Review_Ongoing

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Subject indexing assigned by CRD

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Abnormalities, Drug-Induced; Animals; Organisation for Economic Co-Operation and Development; Rabbits; Rats; Teratogens; Zebrafish

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Details of any existing review of the same topic by the same authors
not applicable

Stage of review at time of this submission

Stage	Started	Completed
Preliminary searches	No	Yes
Piloting of the study selection process	No	Yes
Formal screening of search results against eligibility criteria	No	Yes
Data extraction	No	No
Risk of bias (quality) assessment	No	No
Data analysis	No	No

Versions
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