

The Independent Medicines and Medical Devices Safety Review

Written Evidence

Clinicians, Academics and Others: Hormone Pregnancy Tests

Contents

Professor John Abraham.....	3
Tobias Arndt.....	7
Dr Gottfried Arnold.....	13
Jason Farrell.....	15
Professor Carl Heneghan.....	28
Beate Kirk.....	30
Maria Klein-Schmeink MP.....	34
Professor Tim Lewens.....	35
Jesse Olszynko-Gryn.....	38
Professor Neil Vargesson.....	39

Disclaimer

The statements made and the opinions expressed in response to the Independent Medicines and Medical Devices Safety Review’s (‘IMMDSR’) Call for Evidence and in the video recording of the IMMDSR’s oral hearings are those of the authors. They do not purport to reflect the opinions, views or conclusions of the IMMDSR or its members. The statements and opinions made do not imply the expression of any opinion whatsoever on the part of the IMMDSR concerning the truthfulness, veracity, accuracy or legal status of any statements or opinions made and published on the IMMDSR website. Nor does the IMMDSR accept any legal liability arising from any statements or opinions so expressed and published

WARNING: Please be aware some evidence contains descriptions, pictures and audio of the harm suffered by individuals. Some may find this distressing.

Professor John Abraham

Professor of Sociology, Global Health and Social Medicine, Kings College London.

COI: None declared

Submission to Independent Panel on Review of Medicines and Medical Device Safety

30th October 2018.

Here are my comments on Report of the Commission on Human Medicines Expert Working Group on Hormone Pregnancy Tests (hereafter 'EWG')

On page ix, EWG Report says that when HPTs first became available in the 1950s and early-mid 1960s, 'companies were not legally required to ensure that marketed medicines met appropriate standards of safety and effectiveness' I'm not sure that this is true. One would need to consult a lawyer specializing in consumer law to check this. Even before the Medicines Act, didn't consumers have some legal protection under general consumer product law like a manufacturer's responsibility not to market a defective product and duty of care etc.? This needs to be checked out. It is, of course, true that until 1971 (implementation of the 1968 Medicines Act) that pharmaceutical manufacturers were not legally required to submit to a UK government regulatory body (then the Medicines Division within the Dept of Health) evidence demonstrating the safety and efficacy of their products according to standards established by the regulatory body.

On page 5, EWG Reports says that documents/data were received from Bayer but that some of this must remain confidential to the EWG to protect the interests of Bayer. What steps have been taken to ensure that Bayer have released to the EWG comprehensive sets of data and documents, rather than a biased selection? Under UK pharmaceutical regulation, public safety and the public interest is supposed to take priority over narrow commercial/institutional interests. In this regard, what steps have been taken to allow release of all relevant data into the public domain so that the public interest appropriately over-rides commercial interests?

On page 23, report says that surveillance systems are not designed to test for causation. Indeed, they're not designed to test for causation, but their purpose is to send warning signals based on suspicion of causation. With all these datasets and types, total proof of causation is close to impossible, but there are different strengths of evidence. Same page under 3.2.3, the report rightly points out all the limitations of spontaneous reporting systems. Indeed, and that's why it is so important to have absolutely stringent and rigorous pre-market testing and independent regulatory enforcement and scrutiny of that testing. Yet, I gather that did not happen in this case before the drug was first placed on the market (see below regarding teratogenicity testing in animals).

On page 23 under 3.2.3, Report acknowledges that adverse event reporting suffers from the well-known phenomenon of under-reporting of ADRs by both doctors and patients. The extent of under-reporting has been estimated to be as low as 10% in some studies, and some analysts have even suggested it might be as low as 1% (though probably higher than that for serious ADRs). This means

that there could be many more injuries than actually show up in reports, so quantitative comparison using such data might well underplay any causal association.

On page 34, discussion 4.6 reveals the enormous unknowns and uncertainty involved. In this context, it is better to simply say we don't know what has happened here rather than pretending it implies 'weak evidence'. On page 35, report states that there is 'insufficient evidence to determine whether taking the doses found in Primodos could have had an effect on the developing fetus, via a direct pharmacological action'. But there is also insufficient evidence to determine that the ingredients could not have such an effect. In short, the quality of evidence is too weak to know.

On page 42, the report reveals that the ingredients of Primodos were found to have teratogenic effects in mice. The adverse teratogenic effects were found to be 'drug-related'. This is extremely concerning and indicates a risk of teratogenicity to humans. Moreover, there were many more adverse effects in the high-dosed mice even though there were fewer of them (33) compared with controls (75). Yet the EWG report makes the astonishingly complacent comment (page 42) that it is difficult to put this in context because there were 75 control foetuses compared with 185 test foetuses – a misleading conflation of all three test groups on three different doses into a single total of 185 when of course one compares the control group with each test dose group and indeed each test dose group with each other to determine whether or not an effect is drug-related.

Furthermore, the fact that no clear evidence of teratogenicity was found in rats, rabbits or non-human primates (pages 43-45) provides some reassurance that the positive teratogenicity findings in mice may not extrapolate to humans, but it does not justify the complacent conclusion that the teratogenic effects in mice are species-specific to mice (page 45) because a drug could be teratogenic in one animal species (mice) and humans, while not teratogenic in several other animal species. The fact that the ingredients of the drug were found to be teratogenic in mice is extremely serious indeed for the teratogenic risk assessment of Primodos in humans, particularly in view of the fact that the therapeutic benefit offered by this product was minimal to non-existent.

I further note that Primodos was placed on the market in 1958, but the first cited animal study of the drug's teratogenicity by Schering is 1963 (pages 106-107 of EWG Report). In other words, this implies that Schering placed the drug on the market to be given to people before conducting any teratogenicity testing of the drug! The 1978 and 1979 teratogenicity studies in mice by Schering are discussed showing drug-related adverse effects (page 42 of EWG Report). Did Schering report those findings to the UK regulator (then known as the Medicines Division of the Department of Health?). Where is the discussion of Schering's 1965 teratogenicity studies in mice? (Cited as references only on page 106 of the EWG Report). What was their quality and what did they find? On page 42, the EWG Report merely states: 'Lower doses did not produce an increase in malformations in earlier experiments with mice'. What sort of experiments? Let's see the data. This is highly unsatisfactory.

On page 58, the report says: 'comparison of the pattern of congenital anomaly reports in the offspring of women who were given HPTs' compared with such anomalies in the general population 'showed a higher proportion' (double or more) 'in some anomalies and a lower proportion' (half as many at most) 'in others'. Confronted with these findings, the EWG seems to take an 'averaging out' approach to suggest that overall these findings imply that no conclusions can be drawn. I agree with the EWG that there are major limitations of the data. Such limitations of the data are in capacity to detect a causal connection between exposure and harm. Such limitations lead me to think that it is

quite remarkable that some anomalies have been detected with over double proportions. In particular, the limitations of the data imply that because such data cannot prove causality does not permit the conclusion that there is no causality. Here one might conclude that there is weak or insufficient evidence to conclude that there is not causality.

Regarding a similar comparative exercise on page 58 of report using the MHRA spontaneous ADR reports database, the same type of comments apply. Given the very poor capacity of such a database to detect causality, it is striking that the database detected a higher proportion of 'limb-reduction defects' among the HPT-exposed group. On this basis, one might conclude that there is insufficient evidence to conclude that there is not causality.

On page 58, under 5.2.7, the report states: 'The available adverse event reporting data were limited and do not support a causal association between use during pregnancy of HPTs, including Primodos, and congenital anomalies'. But it has been known for decades that such data is not, and never has been, designed to prove such water-tight causality because there is no experimental design (no prospective control group, little or no control over sampling, and no accurate knowledge of the extent of under-reporting etc.). It is striking, however, that such data has detected higher proportions of some anomalies as a warning. The correct conclusion to draw from the findings from this data is that due to the limitations of the data a causal association between Primodos and various adverse effects (under discussion) cannot be ruled out.

Regarding the epidemiological studies reviewed by the EWG, many, if not all, of these studies had limited capacity to detect and demonstrate a causal association between Primodos and various adverse effects under discussion. Nonetheless, on page 67, the report states that the most robust study of congenital heart defects showed a statistically significant two-fold increased risk of cardiovascular anomalies. Similarly, on page 68, the report states that all five studies of 'limb - reduction defects' showed increased risk (averaging about two-fold), and that the best designed of these studies showed a statistically significant association. Given the limited capacity of these studies to detect a causal association these are quite striking findings. The EWG concludes on the epidemiological studies by saying: 'There is limited evidence for a weak association between the use of HPTs and congenital heart defects, limb reduction defects or oesophageal atresia, which could be due to bias or chance'. In the best studies, the associations reached statistical significance, despite a low capability to do so, so the association may not be appropriately described as 'weak'. To my mind, the epidemiology studies demonstrate that a causal link between Primodos and the adverse effects under discussion cannot be ruled out.

Turning to section 8, on page 95, the EWG correctly states that it is not possible to reach a definitive conclusion on causality due to the nature of the data – that was always the most likely outcome from the outset. The correct conclusion to draw from this, however, is not the one reached by the report, but rather that a causal connection between Primodos and the adverse effects under discussion cannot be ruled out because the available data-set does not have the capability to demonstrate causality even if such causality existed. It only has the capability to indicate risks and (significant) associations, which it has done in some settings, both clinical and non-clinical.

My overall conclusion is as follows. There is evidence of drug-related teratogenicity in mice. There is evidence of some associations between severe adverse events and consumption of Primodos in some epidemiological studies, spontaneous ADR reporting, and in the subsequent reports of those

who claim that they have been injured by the drug. Given all of this (and especially the very severe injuries potentially involved), the therapeutic benefit of this drug to consumers would have to be very strong indeed to merit taking such risks with people's lives. Yet many women took this drug merely as a pregnancy test for which there was no therapeutic need. If it is true that the manufacturer placed the drug on the market in the late 1950s and 1960s without any teratogenicity testing, then, irrespective of legal determinations, the company failed consumers ethically. Government very broadly also failed consumers because it did not introduce formal drug safety regulation earlier, even though the UK Government was well aware of the 1937 elixir sulfanilamide drug safety disaster in the US, which led to the introduction of drug safety regulation in the US (the US 1938 Food, Drug & Cosmetic Act). The UK Government was also far too slow in introducing formal drug regulation after the 1961 thalidomide disaster. The US had strengthened their regulations by 1962. It took the UK Government until 1971 when the 1968 Medicines Act was implemented. Even after regulation backed by law and legislation was introduced, there are questions about how quickly and effectively the UK regulators acted to protect consumers from the risks of taking Primodos as a pregnancy test. Keeping the drug on the market for any other indication should have necessitated an immediate evaluation of the therapeutic advantage it offered over alternative treatments. That advantage would have had to be very considerable to justify the potentially severe risks. I am not aware of any evidence to support such a considerable therapeutic advantage. Without such an advantage, the drug should have been removed from the market immediately by the regulators. Primodos is an example of regulatory failure in the context of scientific uncertainty (about in this case causality of adverse safety events) Failure first of self-regulation within the pharmaceutical industry and then government regulation. What is needed in the first place is a statement by the current Government admitting this. There is then a need for the Government to engage with the company (and of course those claiming to have been injured) in a discussion about how they are going to deliver a just settlement instead of persisting with a misguided quest for definitive proof of causality – there may be some lessons from the practolol disaster in this respect.

Finally, regarding conflicts of interest of EWG members, what exactly were the members asked to declare? Were they asked to declare *both personal and non-personal* financial interests in pharmaceutical companies in general, or just *personal* interests in pharma companies, or just personal and/or non-personal interests in *Bayer/Schering*? Some questions remain here and are not sufficiently clear in the EWG Report.

Tobias Arndt

Researcher, Chief Operating Officer of European Dismelia Reference Information Centre (EDRIC)

COI:

I declare no interest whatsoever in pharmaceutical or medical device industries.

Submission:

Tobias Arndt submitted the following evidence:-

(1) Schering studies and complicity of regulators in Germany from criminal investigation documents at Landesarchive

Schering studies – EE, NETA

Embryotoxicity

EE as abortifacient : EE Testing for embryotoxic effects in rats 17.4.1973 ⁱ

- Severe dose-dependent reduction in the average body weight gain - 0.03, 0.01, 0.3 mg/per kg body weight
- 20 % of all implanted embryos resorbed after 0.1 and more than 50 % after 0.03 mg/kg – Resorptions without fetal remnants – this points to a loss in early pregnancy that could thus be caused by a malformation
- After 0.3 mg/kg: Agnathy of the lower jaw, pig tail, a rudimentary tail and oedematous swelling of the whole body – 4 abnormalities in 88 living foetuses after more than 50% had been resorbed.

11 out of 20 mother animals had vaginal bleedings, three had no living offspring

- CONCLUSION: No need to follow up because product is an ABORTIFACIENT “its intended use for discontinuing pregnancy, (p.c. "emergency medication")

NETA – Rabbits already 29% resorptions after ½ human dose ⁱⁱ

- After the conversion of animal doses to human equivalent doses based on body surface area as recommended by the FDAⁱⁱⁱ this is only 0.16 of the human dose.

EE/NETA in combination as PRIMODOS^{iv}

- After 25HD Rabbits (converted rabbits 8HD, rats 4HD):
 - Rabbits 100% resorptions
 - Rats lower weight gain; anomalies in two foetuses (oedomatic bodies; anophthalmia and erroneous brain development)
- The test with 2.5HD would yield less than the human equivalent dose in both species and thus the absence of findings is insignificant

EE - Preliminary report 773 – for dose determination 28.12.1972^v

- After 0.1 mg/per kg body weight: 50% fetuses died off (24 of 50) – surviving all showed clear signs of retardation. All controls showed normal results.

Methodological problems with the Schering inhouse studies

- Fetuses should have been examined directly after noxious exposure
- Analysis where a fetal remnant could not be determined

Complicity of German authorities BGA

- The department head of the German Health Authorities in charge of supervising Duogynon the German equivalent of Primodos Prof. von Eickstedt:
 - Calls himself and the Health Authority Advocates for Schering - 03.08.78^{vi}
 - Ask Schering to provide “studies which do not yield any statistically significant correlation between the use of sexual hormones in early pregnancy and deformities”^{vii}
 - Argues in a ministry meeting on a market removal against such a measure: “no one believes in a causative relationship between the use of sex hormones and the occurrence of deformities”^{viii}
 - Blames consumers rather than the manufacturer: “*Von Eickstedt philosophized (...) it could be a pregnancy disorder, or it could be a woman who is more interested in the appearance of a period than in an existing pregnancy.*”^{ix}

Schering manipulation of experts

- List expert witnesses for SCL^x

Biased:

- Koller^{xi},
- Wilson^{xii},
- Barnes^{xiii}
- Detering^{xiv}
- Fleming^{xv}
- Haller^{xvi}
- Feinstein^{xvii}
- Nocke^{xviii}
- Roussel^{xix}
- Schaefer^{xx} ^{xxi}

Schering internal assessment of situation

- Dr. Inman informs SCL about a 5:1 risk for malformations associated to mothers that took HPTs. That this proceeding was irregular is hinted by the formulation that “the unofficial way” had been chosen to inform the companies affected.^{xxii}

- Legal situation in Germany and the UK were similar: Given the seriousness of malformations as side effects a pharmaceutical manufacturer has the obligation to remove a drug temporarily (and thus a regulatory body has to enforce it) from the market if only the remote possibility exists that the suspicion could be proven correct until sufficient testing is concluded.^{xiii}

(2) Epidemiological Studies

This document lists epidemiological studies that have statistical significance, hence the interval of possible results does not cross the zero line/ hypothesis (the entire interval of statistically possible results only either supports or does not support an association)

However, every epidemiological study – case controlled or cohort - presented in the final report and to the EWG that has statistical significance favours an association between the use of hormones in pregnancy and malformations of the child.

Statistically significant studies

Nervous system defects

Gaal 1972 (case control)

Sainz 1987 (case control)

Tümmler 2014

Goujard 1977

Goujard 1979

Torfs 1981

Heart defects

Heinonen 1977

Nora 1978 (case control)

Nora 1978 (case control)

Orofacial clefts

Tümmler

Digestive system and abdominal wall defects

Lammer 1986 (case control)

Urinary system defects

Goujard 1979

Tümmler 2014

Genital defects

None

Musculoskeletal defects

Limb reduction defects

Lammer 1986

Other skeletal defects

Tummler 2014

VACTREL

Nora 1975 (case control)

Nora 1975 (case control)

Nora 1978 (case control)

All congenital anomalies

Greenberg 1977 (case control)

Epidemiology is key for detecting safety signals in particular for malformations, where an association is difficult to establish due to the difference of time of exposure and adverse pregnancy outcome. To extend a period of not taking action to establishing further results with further experiments violates the consumers right of health which enjoys higher legal protection than commercial interests of manufacturers.

(3) Important points from the Thalidomide Court decision.

Thalidomide trial decision

To point out: this was existing case law in Germany where the major manufacturer of HPTs Schering was based and acknowledged by Schering Chemicals LTD as similar legal situation in the UK at the – time

Still

P 37/38

In the case of a Health enjoys the higher legal protection than financial interests of the manufacturer

“According to the expert report by Professor Läubli, now and again some time can pass before a suspicion can be proven scientifically. During this period the existence of side effects is still on open question: it can be that the suspicion proves to have been unfounded; but it is also possible that the preparation has the claimed side effects and thus leads to damages of the consumer. In such an undecided case and with opposing interests at play, the risk naturally has to be on one side: in case the suspicion is not confirmed, the drug manufacturer who has taken his preparation off the market or limits its sale by making it available only on prescription or by adding warnings risks financial losses; the consumer, on the other hand, who is not informed about the side effect of a preparation

and not protected by the effective supervision of a physician, risks damage to his health and possibly even his life in case the suspicion is proven. The Grand Division has no doubt that the consumer's interest of not exposing himself to health damage by the taking of a medication has priority over the drug manufacturer's interest in the unrestricted sale of his preparation. Health enjoys the higher legal protection."

Pages 39/40

Importance of severity of health damage: Extremely severe damage such as malformation forces the drug manufacturer to respond even when there exists only the possibility – sometimes even only the remote possibility – that the suspicion might prove to be correct

"The severity of the assumed health damage is of essential importance. The more severe the damage possibly caused by the drug manufacturer's preparation, the quicker he has to respond. In regard to individual cases of light damage it might be justifiable if the drug manufacturer at first attempts to disprove the suspicion by conducting specific tests

- 40 -

and only takes action if this is not possible and a higher probability exists based on numerous reports that the suspicion is accurate. In cases of severe damage, protection measures have to be taken even if the suspicious circumstances are relatively small. Extremely severe damage such as malformation forces the drug manufacturer to respond even when there exists only the possibility – sometimes even only the remote possibility – that the suspicion might prove to be correct."

Page 40

On degree of causality: For less important and more easily replaceable medications a comparatively lesser degree of probability is necessary

"Finally, the therapeutic value of the preparation is also essential. More severe damages are acceptable for therapeutically valuable or even vitally necessary drugs than for less important or easily replaceable medication. Accordingly, the drug manufacturer has to take trenchant measures in the case of the former preparations only in case of a higher probability that the existing suspicion is correct. Now and again it might even be in the interest of the patient – and this always has to be the primary concern – that a drug will continue to be sold (under strict precautionary measures) because of its special therapeutic value despite severe proven side effects. For less important and more easily replaceable medications a comparatively lesser degree of probability is necessary."

Page 41

Drugs have to be labeled with warnings, put under prescription or withdrawn – conditions for the severity of action are severity and frequency of damages, chance of cure, therapeutic value of the preparation

"The answer to the question which measures the drug manufacturer has to take depends significantly on the how the protection of the consumer can be achieved best. Therefore measures of a more internal kind, e.g. the initiation or implementation of animal experiments or the

instruction of an established clinic to carry out specific investigations, are only of secondary relevance for the assessment of this question; these measures aim first and foremost at solving the cause of the side effects and do not have any direct and immediate external effect. Measures which serve the protection of the consumer directly are in essence: sufficient information of physicians and consumers, the introduction of sale on prescription only and the withdrawal of the drug from sale. The question which of these measures is to be considered in an individual case cannot be answered in general because the decision is determined by factors that differ from one case to the next.

The previously mentioned criteria (severity and frequency of damages, chance of cure, therapeutic value of the preparation) are also important. It is obvious that the drug manufacturer has to take the more trenchant measures the more severe and frequent the damages and the less likely the chances of a cure and the therapeutic value of the drug are.”

Schering studies

Dr Gottfried Arnold

Paediatrician (Retired), Germany

COI:

1. I don't have any commercial/financial connections to pharmaceutical or medical devices industry nor any conflict of interest to the Review.
2. As a retired medical doctor I still feel obliged towards the victims of Primidos^o/Duogynon^o without having any financial conflicts.

Submission:

As a German pediatrician I want to give the following comments / questions:

1. Since the publications on Estradiol¹, DES², on DES+Ethisteron³ there is no doubt that natural and synthesised estrogens can produce malformations: feminisation in male and masculinisation in female, all kinds of herma- and pseudohermaphroditism.
2. In the mean time there are further publications on mutagenic and cancerogenic action of these hormones
 - a) the toxicologist Schardein⁴: Ethinylestradiol is teratogen
 - b) the cancer researcher Liehr⁵: estradiol is genotoxic mutagenic carcinogen
3. Please note the embryological fact that a male fetus who is 6-7 weeks⁶ old, about 3 cm, or a few grams of weight is producing his own testosterone (and AMH) in order to rebuild the female and build up the male urogenital organs.

So there is no doubt, there is no question: there are receptors in the very early fetus!

So we can explain easily the urogenital malformations.

4. And what about all the knowledge of the last 20 years on endocrine disrupting chemicals?

Very little amounts – please note the range: $10^{-6-9-12}$ – and the non-monotonic dose-effect-relations lead to the possibility of for instance breast cancer^{7,8}

5. If You think there are some (perhaps not much) genetic disorders which may mimic the hormone effects, you may tell these people, that they get no money. What about all the other people who will have no genetic etiology? These people have to get
 - a) a verification that their problem has become by the early use of hormones in an unbelievable high dose
 - b) a financial compensation

If You want further argument, I would like to give your literature, evidenced- based medicine.

Yours sincerely

Dr. Gottfried Arnold, Germany 

1 Greene, R.R.M.W. Burrill, *Am. J. Anat.* 67, 305 (1940)

2 Kaplan, Male pseudohermaphroditism: report of a case, with observations on pathogenesis. *N Engl J Med* 1959, 261, 641

3 Beral, V. & Colwell, L., Randomised trial of high doses of stilboestrol and ethisterone therapy in pregnancy: long-term follow-up of the children. *Journal of Epidemiology and Community Health*, 1981, 35, 155-160. <https://jech.bmj.com/content/jech/35/3/155.full.pdf>

4 Schardein, J. L., *Chemically induced birth defects*. Marcel Dekker, N.Y., 2000, p. 54 u. 55

5 Liehr, J., Is estradiol a genotoxic mutagenic carcinogen? *Endocr Rev.*, 2000, 21, 40-54. DOI: 10.1210/edrv.21.1.0386

6 <http://embryology.ch/allemand/cgametogen/determ01.html>

7 Soto, AM et al., Does breast cancer start in the womb? *Basic Clin Pharmacol Toxicol.*, 2008, 102, 125-33 <http://onlinelibrary.wiley.com/doi/10.1111/j.1742-7843.2007.00165.x/pdf>

8 Thongprakaisang, S et al., Glyphosate induces human breast cancer cells growth via estrogen receptors. *Food Chem Toxicol*, 2013, 59, 129–136 . DOI: <https://doi.org/10.1016/j.fct.2013.05.057>

Jason Farrell

Sky News

Supplied the following evidence to the Review:-

(1) Primodos – Letter for the Baroness

Dear Baroness Cumberlege,

Thank you again for including me in the process of the review and I understand I'll be meeting you again soon. Following our previous meeting, I was asked to email you a summary of my concerns about the Expert Working Group (EWG) report published in November 2017. Here they are in 13 key points:

1. Presentation to the media

On the day the EWG report was published the media was told in a press conference that the EWG had ended the question over "causal association." It was repeatedly put to journalists that the review's main finding was that there was NOT a causal association between Primodos and malformations. And this therefore was how it was reported.

However, this was a misinterpretation of the report itself. The actual conclusion reads: "The EWG's overall finding is that the available scientific evidence, taking all aspects into consideration, does not support a causal association between the use of HPTs, such as Primodos, during early pregnancy and adverse outcomes, either with regard to miscarriage, stillbirth or congenital anomalies."

Only on reading the report in full do we discover that the "available scientific evidence" is outdated, and therefore not trusted to "support an association." No new research had been commissioned. There is no strong evidence to disprove an association.

It is clear from the report that they don't, and have no scientific ground to say that there is NOT a causal association.

2. Altered conclusion

An October draft version of the report obtained by Sky News said: "On the possible association between exposure in pregnancy to HPTs and adverse outcomes in pregnancy (in particular congenital anomalies, miscarriage and stillbirth) including consideration of any potential mechanism of action. Having reviewed all the available relevant evidence with the benefit of up-to-date

knowledge within the relevant specialisms, the limitations of the methodology of the time and the relative scarcity of evidence means it is not possible to reach a definitive conclusion.

This sentence was removed from the final report in November, after Marie Lyons pointed out in a meeting before publication that “if it was not possible to reach a conclusion, why had they reached a conclusion?” It was at odds with the sentence that came after it, which read: “Nevertheless, based on an extensive and thorough review the EWG's overall finding is that the scientific evidence does not support a causal association between the use of HPTs such as Primodos, during early pregnancy and adverse outcomes.”

When the authors were quizzed by MPs a month later about why the line was removed, chair of the CHM expert group Ailsa Gebbie said: "The report went to the Commission on Human Medicines who had tasked us with developing the report. They all commented on it very fully... They felt we should strengthen the wording, and put a greater clarity on that." This seems like an extraordinary admission that undermines the independence of the inquiry.

Sky News also discovered that in the October draft report a forrest graph showed that the most robust epidemiological studies indicated that there was an association between hormone pregnancy tests and malformations

The graph showed 12 Studies - eight favored an association of which one particularly robust study was statistically significant, 2 were not in favor for an association but none of them statistically significant.

However, a month later when the final report was published this very compelling graph was removed. There has been no explanation for why this was done.

3. “Available scientific evidence” – some of it is missing

I’m not convinced that all available evidence has been considered. For example a doctor called Norman Dean who worked for the Royal College of GPs did an extensive study in 1969. Dean found that when women used hormone pregnancy tests there was a higher incidence of malformed babies, miscarriages, still-births and infant deaths. He said the findings were "alarming" and on 5 March 1969 he wrote to the manufacturers of the drug, Schering, advising them: "Primodos should be withdrawn".

So how did the EWG assess Norman Dean's work? On page 51 it lists where they obtained studies about the drug. In a column marked "UK Royal Colleges" it simply says: "No information available." Norman Dean's study has disappeared.

When we checked the Royal College archives earlier this year we found a handwritten letter from Dr. Dean, which said he had "a bad conscience" about the drug, not on his part, but on behalf of the medical industry. We did not, however, discover the report itself.

When I asked why Dean's study was not available for the CHM experts to consider, the Royal College told me that they were approached to submit evidence in 2014 and "at the time, we were unaware of the unpublished 'Scottish Study' in our archives. Because it was unpublished, and the way archives are catalogued, this wouldn't have come up in any searches".

So, I question whether "available evidence" has been fully considered.

4. Destroyed evidence

Our documentary earlier this year uncovered documents from archives in Berlin.

Here we found that a senior scientist at the Committee on Safety of Medicines conducted a study on women and their babies and in 1975 reported a 5:1 risk of the child being born with malformations if Primodos was used. Dr. William Inman decided to warn the manufacturer of this so they could "take measures to avoid medico-legal challenges". But the public wasn't informed and he later destroyed the material on which his study was based. So again this work was not available for the CHM to consider. Nor do they make reference to any concern about this.

But there is a big concern relating to this. It is clear from our research of private letters that the regulators admitted they had "no excuse for the 8 year delay" in taking Primodos off the market when the dangers became apparent back in 1967. Yet some of these regulators are the same the people producing the epidemiological studies in the mid-1970s that eventually cause the drug to be removed. It seems these regulators had a motive to play down the significance of their findings. There would have been greater public outrage at their earlier decisions if there had been a successful legal challenge against the manufacturer. To me this is why the EWG were blinkered by deciding to "only look at the science" without considering the circumstances in which the science was produced, and the paper trail, which shows a clear cover up of information.

5. Possible Association question remains unanswered

The EWG was asked to see if there was a “possible association,” however they decided to change their own terms of reference to look for a more difficult to prove “causal association.” The reason to look for a “possible association” was because if it existed then it would raise questions about whether the alarm bells were properly responded to by the manufacturer and UK regulators. Was there an assessment of the benefit/risk ratio of Primodos remaining on the market? It can be argued that even if association is not conclusively proven – if a drug raises questions, it should not be the victims living with uncertainty for the rest of their lives about what damage it might have had. The burden of proof should be for regulators to prove the drug is safe. Especially where there is not a strong medical argument to maintain the drugs availability (as can be argued with Valproate, but not with a pregnancy test drug, especially when other methods of pregnancy testing are available). This approach was taken by countries such as Sweden, resulting in Primodos being banned eight years earlier than in the UK.

The CHM says that it was not set up to examine regulatory failings. However, without investigating this we will never get to the bottom of the Primodos story. It is necessary to ask: “was there a potential risk” - and if so – “was it acted on?” The simple question should be: “were the alleged victims let down?”

6. So what is the level of causality?

The EWG do not specify or discuss what level of causality has been examined.

According to our medical researcher Tobias Arndt, there are six different levels of causality:

- a. Strict causality – side effect a) only occurs when there is cause b) there is no cause c) or d) that could cause a). Thus, you observe a syndrome and it must be one specific cause. Even thalidomide is not that strictly causal.
 - i. This is the type of causality that a lay reader would expect and that seems to be evoked here.
- b. Likely causality
- c. Possible causality (thus “possible association”)
 - i. The paper on causality assessment of the WHO's Uppsala monitoring centre says that almost all side effects are (if they are not conditional) somewhere between likely and possible
- d. Unlikely causality
- e. Undeterminable causality
- f. Conditional causality
 - i. This is what the report should have examined if at all a change of the remit would have been justifiable because the report says correctly on page IX: “Today, the cause of the majority of all congenital anomalies remains unknown. Known causes include genetic causes (inherited or occurring without prior family history) and certain medications and medical conditions in the mother. It is likely that many congenital anomalies are caused by many environmental and genetic factors acting together.”

7. Questionable assessment of Epidemiology

When it comes to epidemiology the conclusion seems at odds with the evidence within the report:

Page 67, the report states that the most robust study of congenital heart defects showed a statistically significant two-fold increased risk of cardiovascular anomalies.

Page 68, the report states that all five studies of 'limb -reduction defects' showed increased risk (averaging about two-fold), and that the best designed of these studies showed a statistically significant association.

Page 68 (Greenberg, 1977) best study supports association - "*the comparator group were not women who also sought a pregnancy test*" (This limitation is nonsense see below) For all groups of defects forest graphs show far majority of studies support association (see below)

The report authors assert that because these studies used outdated methods, it could be that the association was overstated. However, a number of experts such as John Abraham of Kings College University and Tim Lewens from Cambridge University have told Sky News that if the methodology was outdated, it is just as likely that the association was understated and causality is actually higher than recorded. Why has the panel only considered the possibility that the association could be less?

One example.

A study done by Dr Olli Heinonen (one of the biggest ever done) in 1977 shows a statistically significant risk of cardiovascular malformation. The EWG says:

"Many studies were judged to be at risk of bias due to: selection bias, exposure misclassification resulting in recall bias and/or lack of control of confounding factors, making the findings difficult to interpret. The study considered to be most robust (Heinonen, 1977) showed a statistically significant two-fold increased risk of cardiovascular anomalies but the plausibility of some of the cases in terms of the timing of exposure was subsequently questioned in other publications (Wilson, 1981; Wiseman, 1984)."

Wiseman was a Schering scientist (makers of Primodos) and his re-evaluation in 1984 argues Heinonen had possibly overstated the risk. The EWG do not mention this reviewer was a Schering scientist nor did they mention that a subsequent independent review by Hook in 1994 actually showed that Wiseman's reexamination was flawed. Hook concluded Heinonen had probably understated the risk. He concluded: "If anything, the quantitative consequences of the Wiseman and Dodds-Smith review of the data, when applied in an unbiased manner, result in an increase in the

measure of effect." The EWG make no mention of Hook's analysis. Could they not have examined the data themselves rather than just dismissing it on the word of a Schering scientist?

Experts we have asked to assess the main body of the report found that all epidemiological studies presented by the EWG that are statistically significant are in favour of an association between hormones and birth defects. Two of these statistical significant studies - Heinonen and Greenberg - are explicitly qualified by the report as robust studies. Of the Forrest graphs included in the report we find the following

Nervous system defects

Overall 15 Studies, 10 favor an association 6 are statistically significant¹, 3 do not favor an association none of them statistically significant,

Congenital heart defects

Overall 15 Studies, 12 favor an association 4 are statistically significant, 3 do not favor an association none of them statistically significant

Orofacial clefts

Overall 5 Studies, 4 favor an association 1 is statistically significant, 1 does not favor an association none of them statistically significant

Digestive system and abdominal wall defects [PAGE 72](#)

Overall 9 Studies, 6 favor an association 1 is statistically significant, 3 do not favor an association none of them statistically significant

Urinary system defects

Overall 3 Studies, all 3 favor an association and are statistically significant, no study that does not favor an association.

Genital defects

Overall 2 Studies, all 2 favor an association however none of them statistically significant

Limb reduction defects

Overall 5 Studies, 5 favor an association, of which 1 is statistically significant, no study that does not favor an association.

Other musculoskeletal defects

Overall 3 Studies, **1 favors an association and is statistically significant**, one is completely insignificant and one does slightly not favor an association but is not statistically significant.

VACTERL

Overall 5 Studies, **3 favor an association and are statistically significant**, two completely statistically insignificant

All Congenital anomalies

Overall 12 Studies, **8 favor an association of which 1 particularly robust study is statistically significant**, 2 are not in favor for an association none of them statistically significant.

It seems odd to conclude the association was unsupported by the science. The science does support an association – the EWG appear to mistrust the science. However, part of the reason for this is that the EWG make out-dated assumptions themselves.

8. A Key Unsupported Assumption

There is an unsupported assumption in the EWG study that women who took a pregnancy test were different to women who did not.

Page 25 “A key bias is in comparing those who sought a pregnancy test compared with those who did not seek them.” **Page 60** “This is because the risk of adverse outcomes, including congenital anomalies, in women who choose to have a test will differ from those who chose not to.” **Page 68** (Greenberg, 1977) (...) “the comparator group were not women who also sought a pregnancy test”.

This is nonsense – and the EWG were told so by an expert in the history of pregnancy tests Jesse Olszynko-Grin. From the 1960s and later, pregnancy tests were not done because of concerns about a problem. On the contrary, when contraception and pregnancy tests arrived in the 1960’s and 70s, many women adapted their behaviour to ensure positive pregnancies. There is no evidence that women who sought to find out whether they were pregnant were therefore more likely to give birth to a child with malformations. Indeed of the dozens of women I have interviewed who had a child with a birth defect after taking Primodos – none of them went to the doctor because they feared complications. Also, it was the doctor who decided to give them Primodos – they didn’t ask for it.

In the November press conference I asked panel for evidence to support this assumption; that women who sought clarification of pregnancy were in a high-risk group. I was given none.

Later, I did however find letters in German archives sent by Schering to potential supporters of their legal case in 1982 asking experts to adopt this argument. So I am quite shocked to find it as an accepted fact within the 2017 EWG report.

9 – Worrying animal studies

There is no reference in the EWG report to the potential bias in the animal studies based on the fact that many were produced, by Schering, in the knowledge that the company was facing legal action, and that these were tests that should have been conducted a decade ago before giving the drug to pregnant woman to swallow.

The company had been told by its lawyers it was “in neglect of duty” for not conducting the tests earlier. So this would have added further pressure.

However, one 1979 study examined by the EWG - released by Schering – admits that mice were deformed by compounds within the drug.

It reported "visceral malformations, including the heart, lung and thorax wall" and said: "the increase in these malformations in this study should be considered drug related".

I raised this in the press conference and was told it was possibly because the mice were given a higher dose (relative to body mass) than to humans. However, Medical expert Tobias Arndt makes the point:

- A.) We don't know if this is about high doses, the mechanism and metabolism isn't known. Also cofactors are not known.
- B.) Also there is evidence of malformations at low dose. Page 41 of the report shows “equivocal increase in malformations in one rabbit study (Schering #2300, 1976 -two fetuses with umbilical hernia) at doses higher than those used in HPTs. “Omits that dose was only 1/3 HED (Human equivalent dose – which is calculated on basis of the body surface and not body weight).

During the press conference I pointed out that test on rabbits was given at a lower dose than the equivalent taken by humans and indicated skeletal problems and "wavy ribs" caused by the drug.

I was told that the drug's impact might be "species specific." i.e. it could affect rabbits but not humans. This is hardly reassuring. Thalidomide is species specific – some animals such as rats are not affected by it – but humans are.

Furthermore the report makes clear that numerous animal studies found embryos, were killed by the components of Primodos. Indeed the EWG concludes:

"Death of the developing embryo with high doses of estrogens has been consistently observed in animal studies and is now considered to be a well-established effect. A similar effect has been observed in studies with norethisterone (or related progestogens). As may be expected, the combination of norethisterone and ethinylestradiol also showed consistent embryo-lethality in different animal species."

So the EWG accepts that the components of Primodos, whether separate or combined, are embryo-lethal to animals. However, it is clear that the scientists who conducted these tests have not examined whether the dead embryos died because the drug had caused deformities to the embryo. Embryologist Neil Vargusson makes this point –that a number of the babies might have died in the womb *because* they had been deformed. These embryos are then not counted in the assessment of whether the drug caused deformities.

The EWG concluded that the animal studies provided "insufficient evidence" for a connection between Primodos and deformity. However, it can easily be argued that these studies offered no reassurance that the drug was safe.

10 - Why not include the most up-to-date study?

The Committee on Safety of Medicines wrote to doctors in November 1977 warning them that Primodos could cause malformations saying: "the association is confirmed." You will note that the language here is unequivocal: it was "confirmed".

The 2017 EWG study is at odds with conclusions of 1977, but it's also important to note that, because Primodos is no longer in use after 1978, very little scientific research on the compound has been produced since then to counter the view that the medical committee took. The EWG did not commission new studies, they simply reviewed "available" studies, mostly decades old and, it seems, decided these were not very good - the science was poor and therefore "didn't support" a causal association.

However, a very recent study was done in Aberdeen in 2016/17. Embryologist Dr Neil Vargusson presented his preliminary findings to the EWG. It is extraordinary then that the EWG dismissed this study because it had not been peer reviewed or published.

This is especially strange as the EWG paper included 44 older studies, many produced by the manufacturer Schering, which had also not been peer reviewed or published.

Page X “A number of published studies (a total of 38) and unpublished studies (a total of 44) were therefore evaluated to see if there was any evidence for a teratogenic effect with norethisterone or ethinylestradiol.”

Also many of them were subject to bias because they had been produced in-house, in preparation to defend legal action.

Having complained about the outdated nature of the methodology used in studies four decades ago – why did the EWG not wait a couple more months for an up-to-date one?

Dr Vargusson’s study was submitted for peer review at the time the EWG report was published in November 2017. His study into the effect on fish embryos, published three months later February 2018, showed how the drug could deform numerous aspects of the embryo development and these corresponded with the types of complaints alleged in humans. One thing Vargusson did, that earlier studies had not, was to show that the effect of the drug was dependent on when it was administered during development. The earlier – the more damage.

He concluded that Primodos “has the potential to deform embryos” in humans. In an interview with Sky News the man who has conducted the first study on Primodos in decades said: “No-one could put their hand on their heart right now and say this didn’t do this.”

11. Misleading Endorsement of EWG report

Thalidomide campaigner Nick Dobrik was an expert asked to sit in on the EWG investigation to oversee the workings of the group.

The panel, the Department of Health and even the Prime Minister, used his name to give credibility to the report after its publication.

On the day of publication the press were told Mr Dobrik had endorsed the review and supported the conclusions. This was in fact a misrepresentation.

He told Sky News he was "very angry" about this and he did not agree with the conclusions. In an interview he described it "as a whitewash." He said: "I think the decision of the committee was plainly and simply wrong. They had no right to reach the conclusion they did. The victims have been let down."

12. Poor research

The report makes some badly researched points. For example it states that in 1978

"because the alternative non-hormonal pregnancy tests were becoming more widely available, the products were withdrawn from the market by the manufacturers."

This is not true. Internal documents show they fought tooth and nail to keep it on the market believing that a withdrawal would be "an admission of guilt." They were forced to withdraw the product by UK regulators.

The EWG also writes:

- "In 1970 Schering removed the indication 'diagnosis of pregnancy' from the Primodos datasheet, stopped promoting Primodos for pregnancy testing, and stopped providing free samples to healthcare professionals."

This is inaccurate. Evidence we've seen suggests they took the indication off the datasheet in 1974, and notes from their sales department suggest they were still promoting it as a pregnancy test drug in the mid 1970s. Indeed sales of Primodos as a Pregnancy test drug rose in the UK by 10,000 between 1970 and 1971. And even in 1974, 120,000 women used the drug as a pregnancy test. How could this happen if the above statement was true.

Page 23 "*Well recognised limitations of spontaneous suspected ADRs include that there is a variable and unknown degree of under-reporting*" This fails to stipulate that there are studies on the degree of underreporting: level of underreporting at 1-10% - thus only 1-10% of ADR are reported. This is significant when considering the results of some epidemiological studies.

Page ix "*companies were not legally required to ensure that marketed medicines met appropriate standards of safety and effectiveness.*" This is not true: consumers had legal protection under general consumer product law like a manufacturer's responsibility not to market a defective product and duty of care. It also makes little consideration of the heightened concerns raised by the Thalidomide scandal and the 1968 Medicines Act which included a process for removing potentially harmful medicines.

Finally on future safeguarding Tobias Arndt notes that “The report does not even know that there is a European Reference Network on ‘rare congenital malformations’ coordinated by an NHS trust in Manchester that has the scope to build a patient register setting the infrastructure for an early warning system throughout Europe – thus including much more data and consequently higher probability of detecting signals much earlier than in a national model.

13. Implications for Medicines today

Page XX: “On whether the Expert Working Group’s findings have any implications for currently licensed medicines.”

“The findings of the review for HPTs, including Primodos, on a possible association between exposure in pregnancy to HPTs and adverse outcomes in pregnancy do not have implications for any currently licensed medicines. They are in fact considered to be reassuring for women who may inadvertently become pregnant whilst taking these hormones for contraception or gynaecological indications.”

Many experts we’ve spoken to disagree with this point and some have described it as misleading and dangerous. Interestingly this statement was put into in the final report in November but was not in the October draft. Why?

How does this work alongside for example the statement on page XV: “Death of the developing embryo with high doses of estrogens has been consistently observed in animal studies and is now considered to be a well-established effect. A similar effect has been observed in studies with norethisterone (or related progestogens). As may be expected, the combination of norethisterone and ethinylestradiol also showed consistent embryo-lethality in different animal species. This effect was dose-dependent and varied according to when and for how long during pregnancy it was given. The mechanism for this effect in animals is not established but may relate to disruption of the relationship between the mother’s hormones that are required to maintain pregnancy and the developing embryo or fetus.”

Considering the above 13 points, I would conclude that there is very little in this report that offers reassurance to women who inadvertently took the drugs while pregnant today. Neil Vargusson says there is “no way” he would give Primodos to a pregnant woman today. For the many reasons listed I conclude that the EWG report is flawed and misleading. Which is hugely disappointing considering the years that campaigners waited for it – and the level of cover up and deception they have experienced from authorities in the past.

Note: In the above have included or paraphrased thoughts or comments made to be by experts such as Neil Vargusson (Embryologist, Aberdeen University), Tim Lewens (Professor of the History of Medicine at Cambridge University) John Abraham (Professor of Sociology of Pharmaceuticals at Kings College London) Jesse Olszynko-Grin (Wellcome Trust Research Fellow). But in particular I have incorporated notes from Tobias Arndt (Medical Author) who has helped me study the report.

(2) Correspondence with Bayer

The IMMDS Review does not currently have permission to publish these files.

(6) Jason Farrell, Sky News. 22 March 2017. Primodos: The Secret Drug Scandal.

Available at <https://www.youtube.com/watch?v=7ZRkCNUQvBA>

(7) Footage of interviews

Extracts available at <https://news.sky.com/story/new-study-links-pregnancy-drug-primodos-to-birth-defects-11565112>

Professor Carl Heneghan

Professor of Evidence-Based Medicine, University of Oxford

Hormone Pregnancy Tests

Conflicts of interests: I declare that I am Director of the Centre for Evidence-Based Medicine (www.cebm.net) at the University of Oxford, a clinical advisor to the All-Party Parliamentary Group (APPG) on surgical mesh. I have received expenses and fees for my media work, and grant funding from the NIHR, the NIHR School of Primary Care Research and the NIHR BRC Oxford, and I am an NIHR Senior Investigator and Director of the NIHR SPCR Evidence Synthesis Working Group. I have received financial remuneration from an asbestos case and given free legal advice on mesh cases. I work as an NHS GP work in urgent care, and I am Editor in Chief of BMJ Evidence-Based Medicine.

The project received funding from the Evidence Synthesis Working Group funded by the National Institute for Health Research School for Primary Care Research (NIHR SPCR) [ProjectNumber 390]. The views expressed are those of the author(s) and not necessarily those of the NIHR, the NHS or the Department of Health.

Executive summary:

We performed a systematic review and meta-analysis that shows that use of oral HPTs in pregnancy is associated with increased risks of congenital malformations.

Evidence that this conclusion is based on:

Background: Oral hormone pregnancy tests (HPTs), such as Primodos, containing ethinylestradiol and high doses of norethisterone, were given to over a million women from 1958 to 1978, when Primodos was withdrawn from the market because of concerns about possible teratogenicity. We aimed to study the association between maternal exposure to oral HPTs and congenital malformations.

Methods: We have performed a systematic review and meta-analysis of case-control and cohort studies that included data from pregnant women and were exposed to oral HPTs within the estimated first three months of pregnancy, if compared with a relevant control group. We used random-effects meta-analysis and assessed the quality of each study using the Newcastle–Ottawa Scale for non-randomized studies.

Results: We found 16 case control studies and 10 prospective cohort studies, together including 71 330 women, of whom 4209 were exposed to HPTs. Exposure to oral HPTs was associated with a 40% increased risk of all congenital malformations: pooled odds ratio (OR) = 1.40 (95% CI 1.18 to 1.66; $P < 0.0001$; $I^2 = 0\%$). Exposure to HPTs was associated with an increased risk of congenital heart malformations: pooled OR = 1.89 (95% CI 1.32 to 2.72; $P = 0.0006$; $I^2 = 0\%$); nervous system malformations OR = 2.98 (95% CI 1.32 to 6.76; $P = 0.0109$ $I^2 = 78\%$); gastrointestinal malformations, OR = 4.50 (95% CI 0.63 to 32.20; $P = 0.13$; $I^2 = 54\%$); musculoskeletal malformations, OR = 2.24 (95% CI 1.23 to 4.08; $P = 0.009$; $I^2 = 0\%$); the VACTERL syndrome (Vertebral defects, Anal atresia, Cardiovascular anomalies, Tracheoesophageal fistula, Esophageal atresia, Renal anomalies, and Limb defects), OR = 7.47 (95% CI 2.92 to 19.07; $P < 0.0001$; $I^2 = 0\%$).

Conclusions: This systematic review and meta-analysis shows that use of oral HPTs in pregnancy is associated with increased risks of congenital malformations.

Heneghan C, Aronson JK, Spencer E *et al.* Oral hormone pregnancy tests and the risks of congenital malformations: a systematic review and meta-analysis [version 1; referees: awaiting peer review]. *F1000Research* 2018, **7**:1725 (<https://doi.org/10.12688/f1000research.16758.1>)

Why do are results differ from the independent Expert Working Group (EWG) assessment?

On the basis of cohort and case-control studies in exposed women, the EWG found “limited evidence for a weak association between the use of HPTs and congenital heart defects, limb reduction defects, and oesophageal atresia,” but “the weak associations that were observed could have occurred by chance or confounding.” [5, p. 69, reference 1]

‘Establishing causal associations in the absence of randomization can be difficult. However, the lack of randomized trials in our analysis should not be seen as a hindrance. It would have been unethical to randomize individuals to drugs with known concerns, and randomization, like systematic reviews, was not the norm at the time. Furthermore, for questions about harms, the Oxford CEBM levels of evidence puts systematic reviews of case-control studies on a par with systematic reviews of randomized trials.’

‘A significant strength of this current study is its use of standard systematic review methods. By asking a focused question solely on exposure to HPTs, and excluding exposure to other hormones, we have been able to assess the heterogeneity of the effect estimates. However, as with any observational studies, there is always the possibility that an unknown confounder could be the cause of the observed difference. While such a possibility cannot be ruled out, the lack of heterogeneity means that such a confounder would potentially have to act in the same direction, despite many different confounders being collected and controlled for. Confounding factors with variable effects on the effect estimates would have probably led to a high degree of heterogeneity, which would have prevented pooling; this was not the case.’

References

[1] Report of the Commission on Human Medicines’ Expert Working Group on Hormone Pregnancy Tests. GOV.UK. <https://www.gov.uk/government/publications/report-of-the-commission-on-human-medicines-expert-working-group-on-hormone-pregnancy-tests> (accessed Oct 2018).

Submitted by:

Carl Heneghan, BM, BCH, MA, MRCGP, DPhil (Oxon)
Professor of Evidence-Based Medicine
Centre for Evidence-Based Evidence,
Nuffield Department of Primary Care Health Science
University of Oxford
[REDACTED]

Beate Kirk

Pharmacist and Pharmacy Historian, Germany

COI:

I have no commercial/financial/legal connection or interest in the pharmaceutical and medical devices industry sector or any other body or organisation of interest to the Review.

Submission:

Speech by B. Kirk at the Bayer AGM on 25.05.2018

Information about the Bayer AGM:

<https://www.bayer.de/de/hauptversammlung-2018.aspx>

Registration of speech on the topic of "gynaecological drugs"

Personal registration on 25.05.2018 at 9:00 am.

Due to a misunderstanding, on 25.05.2018, I was not called upon "as per ordinary procedure" when it came to the speeches.

Herr Wenning has been kind enough – after I pointed this out – to reopen the "speaker block", so that I too can have a chance to speak. Hence my preliminary remark.

Speech by Kirk on 25.05.2018.

Preliminary remark:

Ladies and gentlemen,

I am going to speak on the subject of gynaecological medicines, and I am not surprised that I was forgotten, because this topic is probably not of great interest to the Bayer company. But fine...I'll now give my original speech.

"Paracelsus would be doubled over with laughter."

Dear Sir/Madam,

My name is Beate Kirk and I'm going to talk about Duogynon and the Mirena hormonal coil.

Dear Mr. Baumann,

My question to you, as a leading representative of your research-based pharmaceutical company, is:

Is what you personally said to Dr Arnold's questions on the Duogynon affair in all seriousness the state of teratological knowledge at Bayer?

I'm a pharmacist, I'm a pharmaceutical historian, and I'm shocked.

And I personally consider the statements made by Bayer to be utterly beyond belief.

I do not wish to go over the Duogynon affair again.

Thanks to the speeches, publications and legal disputes of previous years, it is no doubt familiar to you.

On 13th February 2018, the research results of the working group under the Aberdeen scientist Dr Neil Vargesson were published in "Nature".

Vargesson has been able to cause deformities in zebrafish using the ingredients of Duogynon tablets. These animal experiments have confirmed the experiences gleaned in humans.

Incidentally, this is a kind of analogous case to the replication of "thalidomide"-typical deformities by Somers in animal experiments on rabbits.

Your company was asked to comment on the Duogynon affair and the Aberdeen research results. Its answer, in a gist: this does not prove anything, we need animal experiments on primates. In principle, experiments on eligible laboratory animals cannot be transferred to humans.

At first I could hardly believe this statement from Bayer.

At Bayer, in particular, it should be well known that:

"It is the dose that makes the poison."

These are the facts:

Thanks to contemporary eyewitness reports, it is well known that Duogynon was used in the 1960s and 1970s as an abortifacient by taking an overdose. Taking 4 or 6 tablets instead of the 2 recommended in the instructions for use resulted in the abortion of the pregnancy.

How is this possible if it is completely harmless?

Paracelsus would be doubled over with laughter.

As a logical conclusion of the research results from Scotland, British Prime Minister Theresa May and British Health Secretary Jeremy Hunt informed the British Parliament in February 2018: there will be a reassessment.

In Britain, it seems everything will now be addressed.

And in Germany?

How is the "Duogynon affair" playing out in this country?

This is partly in your hands, ladies and gentlemen, shareholders.

The Bayer company can look back on years of experience in matters concerning the effects of drugs on the unborn child. In the Red Lists from the years 1969 to 1980, the drug Cyren, with the ingredient DES, was consistently included in the product range of the Bayer company. As concerns

experiences with DES-containing drugs, I would like to make reference to the report of the Federal Government from 30th October 1990.

In the case of Duogynon, representatives of the "generation of children" have been striving to cast light onto darkness since 2009. But the Bayer company has so far insisted on the statute of limitations.

Ladies and gentlemen,

Drug "disasters" are as a matter of principle not statute-barred.

And so in the UK, everything is now being thoroughly re-examined.

This work to throw light on the matter must also be carried out here in Germany.

With or without the support of the Bayer Group.

For you, as members of the Executive Board and as shareholders, the former would be better. Finally acknowledge your responsibility.

At this point, I would be pleased to wish you my best regards. Those affected continue to fight and have founded the association Netzwerk Duogynon (the "Duogynon Network"). The story continues.

One current medicine from Jenapharm/Bayer seems to cause similar problems to Duogynon: the Mirena hormonal coil, using the ingredient levonorgestrel.

Dear Mr. Baumann,

In the information to gynaecologists, it is strongly encouraged that, should a pregnancy occur while "the Mirena" hormonal coil is in situ, the coil be removed, despite an increased risk of miscarriage.

The reason given is the risk of virilisation, that is, masculinisation, of female fetuses.

Dear Mr. Baumann,

It's about girls. Do you personally not care about their fates?

Pregnancies while using the hormonal coil are in no way rare.

The press reported on the "Baby Dexter" case in 2017.

Google it. Whether Dexter will suffer long-term complications as a result of 9 months of levonorgestrel input from the hormonal coil in the placental tissue of the uterus right alongside him, is something that only his life will reveal.

As a pharmaceutical historian and pharmacist who is familiar with both the Thalidomide and Duogynon cases, I am appalled that a publicly traded company like Bayer has become set on ignoring the matter and sitting it out.

I personally find this scandalous, and suboptimal for Bayer too in the long run.

I plead that this Board not be let off, under any circumstances.

Thank you for listening.

Maria Klein-Schmeink MP

Bundestag MP and Green Party spokeswoman on health affairs

COI:

I have no commercial/financial/legal connection or interest in the pharmaceutical and medical devices industry sector or any other body or organisation of interest to the Review.

Submission:

Berlin, 22/10/18

Call for Evidence for the Independent Medicines and Medical Devices Safety Review

Dear Sir or Madam

I am writing to you to let you know that not only the British House of Commons is currently revising the Primodos/Duogynon case but that the German Bundestag as well is occupied with several parliamentary processes concerning that matter.

Since the beginning of 2017, several petitions have been submitted to the German Bundestag by persons affected by the case in one way or the other. All alike, they demand to clarify the state of affairs, to initiate a round table, or to establish a compensation fund.

It has become obvious that the former Bundesgesundheitsamt (BGA) did not always fulfil its duties in drug safety to the fullest effect. Moreover, there are reasonable grounds to suspect that the interests of the pharmaceutical industry were put over those of patients and drug users at the time.

Investigating those entanglements between the Bundesgesundheitsamt (BGA) and Schering, the Green Party faction has just launched a parliamentary enquiry (Kleine Anfrage) for which my office is in charge. We ask the German government, among other things, to comment on findings in the Landesarchiv Berlin files. Please follow this link to view the document:

<http://dipbt.bundestag.de/extrakt/ba/WP19/2406/240607.html>

I will forward you the answer as soon as we receive it, presumably in mid-November.

We are keen to resolve the Primodos/Duogynon controversy at last. We hope that your review as well as our engagement will help to do justice to all patients affected.

Please do not hesitate to contact me for any further questions.

Yours sincerely,

Maria Klein-Schmeink
(Bundestag MP and Green Party spokeswoman on health affairs)

Professor Tim Lewens

Professor of Philosophy of Science, University of Cambridge

Comments to the Independent Medicines and Medical Devices Safety Review

COI:

I have no current financial/commercial/legal interests in the pharmaceutical or medical devices sectors. I am currently an unpaid external member of an Astra Zeneca advisory committee, and I did occasional paid consulting work for pharmaceutical companies between 2005 and 2010.

Comments:

The main theme I would like to stress in these comments is a regrettable, and potentially misleading, slippage in recent communications regarding Primodos. This is the slide from an assertion about absence of evidence that Primodos has effects threatening to health, to either an implied or more explicit claim about evidence that Primodos has no such detrimental effects. In contexts like these, absence of evidence is not evidence of absence.

Consider the (October 2017) *Report of the Commission on Human Medicines Expert Working Group on Hormone Pregnancy Tests*. The report's main conclusion is this:

The EWG's [Expert Working Group's] overall finding is that the available scientific evidence, taking all aspects into consideration, does not support a causal association between the use of HPTs, such as Primodos, during early pregnancy and adverse outcomes, either with regard to miscarriage, stillbirth or congenital anomalies. (p. 100)

This assertion that research 'does not support a causal association' is ambiguous. On one reading (encouraged by much of the analysis of the report), it simply means that there is not enough evidence to be able to claim with any confidence what the causal role of HPTs may have been with respect to adverse outcomes. On another reading (suggested by some comments in the report) it means something very different, namely that evidence indicates there is not a causal link between HPTs and adverse outcomes. Needless to say, these claims are importantly different: the first simply highlights a lack of robust information, the second claims some reasonable degree of knowledge of HPTs safety.

The EWG's conclusion appears to rest on its repeated claims about the poor scientific quality of the studies that have been conducted on Primodos's effects, and the unreliable nature of other forms of available data that might be used to infer claims about causation. We find many of these remarks: 'Under-reporting...makes it impossible to calculate an incidence rate or absolute frequency for an ADR [Adverse Drug Reaction]' (p. 23); 'being carried out in the 1950s to 1970s, the design, conduct and quality of the studies [surveyed by the ERG] were largely poorer than would be expected of those conducted today' (p. 24); 'The validity of such extrapolations [from in vitro and animal models] is unknown' (p. 34); 'the limitations of the data do not allow firm conclusions to be drawn' (p. 58); 'The design

and methodological rigour or many of the studies identified for review was not consistent with today's standards' (p. 62); poor study design 'made it difficult to draw robust conclusions: that is, the evidence from many of these studies was insufficiently strong to demonstrate either that there was a causal association between HPTs and congenital anomalies or conversely that there was no possibility of a causal association' (p. 69); etc., etc.

What the EWG argues, in other words, is that research has not been of a sufficiently high standard until now for us to say anything with confidence about Primodos's effects. Reliance on these past studies still leaves us uncertain about whether those effects were harmful. When the EWG claims that evidence 'does not support a causal link', it seems this means that, because of poor study design, the evidence gathered to date is not of a high enough quality to provide strong support to any claims about the causal effects of Primodos. Available evidence does not support a causal link, but equally it does not support there being no causal link between Primodos and adverse outcomes. It simply leaves the matter open until better designed studies appear. The EWG's report should have been clearer on this matter, because the choice of words used to state its main conclusion is highly likely to mislead readers by virtue of its ambiguity.

It is hard to understand why the EWG claimed that the group's findings 'are in fact considered to be reassuring for women who may inadvertently become pregnant whilst taking these hormones for contraception or gynaecological indications' (p. xx). If study design thus far has been too weak for conclusions about HPTs' effects to be stated with confidence, then in what sense should women feel reassured? On the face of things, women are simply still in the dark.

Perhaps the EWG did take the view that the available evidence was strong enough to make a causal link between Primodos and birth defects highly unlikely. If that was the case, then the nature of its reasoning—how it progressed from a series of claims about poor study design to a comparatively confident conclusion regarding the absence of an effect—should have been spelled out in much greater detail. In informing women that they 'should feel reassured', the EWG makes a claim designed to comfort women, without showing what (if anything) justifies that claim.

There are similar slippages indicated in the minutes of the more recent ad-hoc EWG meeting on Zebrafish (minutes from 5th October 2018 meeting). This group has discussed a paper by Brown et al 2018, which is acknowledged to be a generally professional study. The group concludes that there are 'knowledge gaps...and that information on the pharmacokinetics, pharmacology and phenotypes of the responses would be required to fully elucidate the translational relevance of this data to humans.' This claim is entirely reasonable: *fully* elucidating translational relevance is a very high bar indeed, and it is inevitable that further knowledge will be needed before it can be cleared. But the minutes indicate a swift move to the much stronger claim that the study 'does not raise any new safety concerns for products in clinical use...'. It is one thing to acknowledge that the study needs to be read in the context of existing evidence, and that its significance may be as yet unclear, another to claim quite so strongly that it raises *no* new concerns.

I am concerned that some of the background assumptions feeding into these review processes have been shaky. For example, David Mowat (Undersecretary of State for Health) told Parliament on October 13th 2016 that: 'if, when the expert group reports next spring, it finds a clear causal link, that will be the time to take further action on issues such as regulation and liability, and everything that goes with that. The first step we are taking is to establish the science.' It is a part of common sense, though, that even when causal links are not established in a clear way—indeed, even when they are highly questionable—it can still be reasonable to take regulatory action in a precautionary manner. This is especially true when the value of a technology is in question, and when safe alternatives are available.

When the effects of a drug, a diagnostic test, or a treatment are uncertain, its use can only be justified by considering, among other things, the potential benefit it brings, and also the availability of alternatives with better established risk profiles. The EWG report says very little about the nature of the positive medical case made for women to use Primodos, and what it does say implies that it was given out on an exceptionally casual basis with no proper consideration of medical need. The EWG notes that 'The members [of the Association for Children Damaged by Hormone Pregnancy Tests'] confirmed that the HPT had been taken within the critical period for fetal development, and that in many cases a test was recommended by the doctor rather than requested, that pills were given to first time mothers who did not consider themselves to be in any high-risk category, and that the doctor in several cases had taken what appeared to be free samples from a desk drawer, rather than making out a prescription' (p. 50). Further evaluation of how the medical case for using Primodos was established is still needed.

Professor Tim Lewens
24th October 2018.

Jesse Olszynko-Gryn

Wellcome Trust Research Fellow, Department of History and Philosophy of Science, University of Cambridge

COI:

I have no commercial/financial/legal connection or interest in the pharmaceutical and medical devices industry sector or any other body or organisation of interest to the Review.

Submission

Evidence provided as part of the Association for Children Damaged by Hormone Pregnancy Tests

Additional evidence provided:

Olszynko-Gryn, J. et al (2018) A historical argument for regulatory failure in the case of Primodos and other hormone pregnancy tests. *Reproductive Biomedicine & Society Online* 6: 34-44

<https://doi.org/10.1016/j.rbms.2018.09.003>

Professor Neil Vargesson

Chair in Developmental Biology, School of Medicine, Medical Sciences and Nutrition, University of Aberdeen.

COI Statement:

I have no conflict of interest with the pharmaceutical and medical devices industry sector. I have been funded (presently or in the past) by The Royal Society, Wellcome Trust, BBSRC, Newlife, NIH, University of Aberdeen, consulted as an expert scientist to lawyers for thalidomide survivors, consulted with thalidomide survivors and alleged Primodos survivors about my research, and have advised international and national Government agencies about my research. We have had no funding from industry, charity nor Govt organisation for our Primodos research (this came from funds from University of Aberdeen).

Studies of Norethisterone and Ethinyl Estradiol (Primodos) in zebrafish embryos

MHRA *ad hoc* Expert Working Group on Zebrafish – London 5 October, 2018

Prof Neil Vargesson

School of Medicine, Medical Sciences & Nutrition

Institute of Medical Sciences

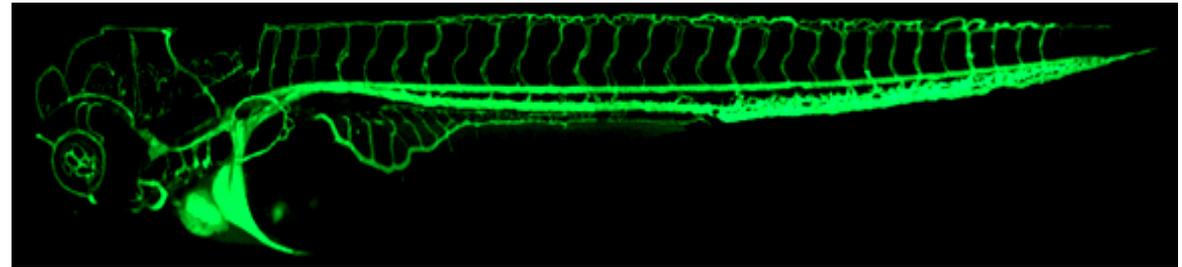
University of Aberdeen

Vargesson Lab

Focused on vascular development and its roles in development, birth defect and regeneration.



Thalidomide
Primodos (NOR/EE)
Clubfoot
Limb regeneration
Phocomelia
Joint formation

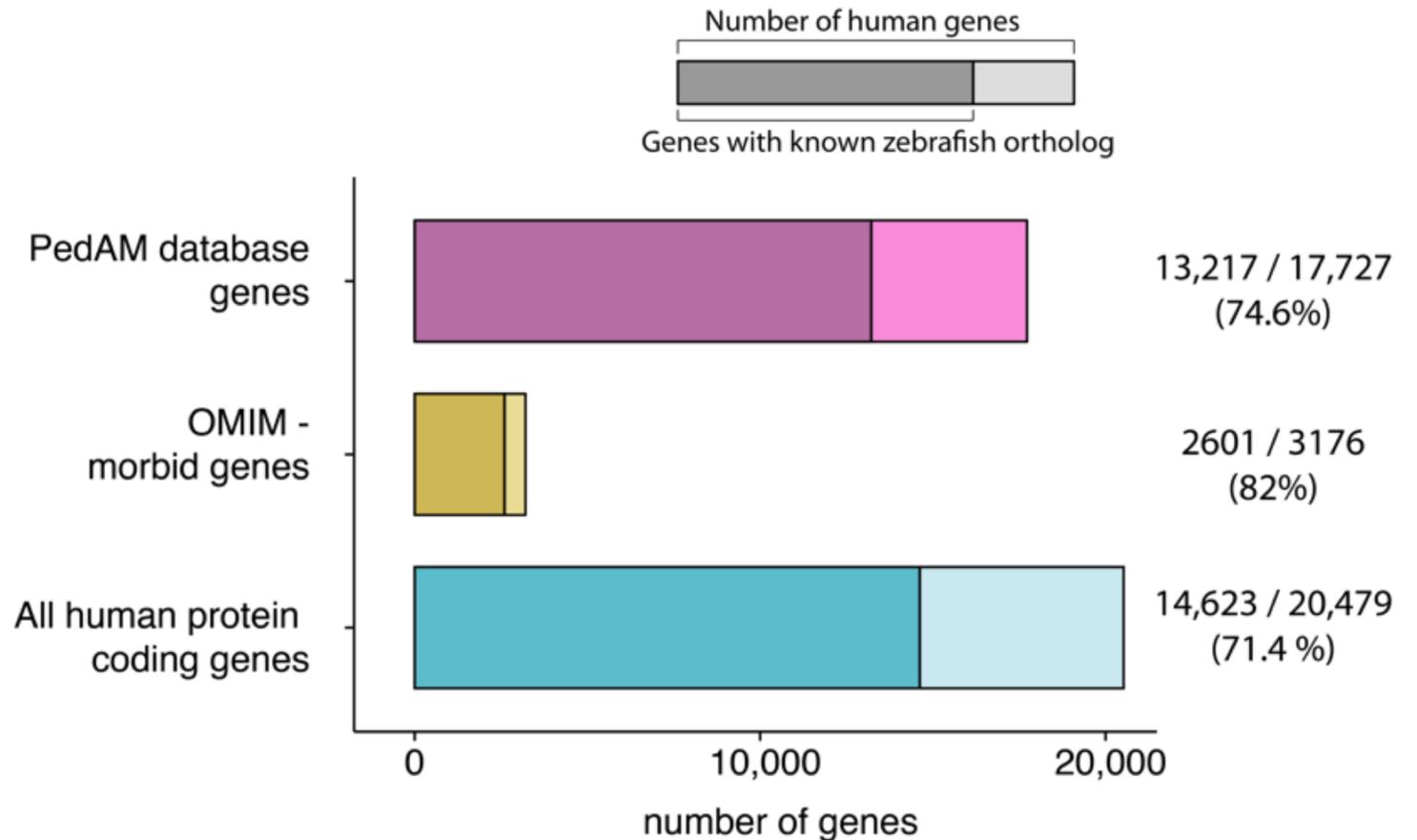
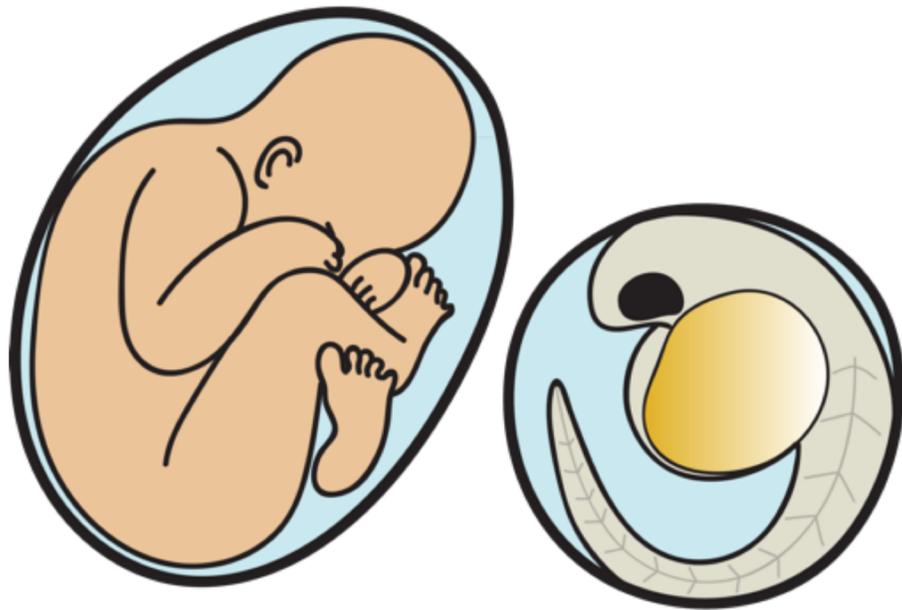


Thalidomide
Primodos (NOR/EE)
Drug screening
Fin regeneration

Early in development embryos of different species look very similar.

Fish, chick, mouse used as model systems to study human diseases – as genes/processes used for development are remarkably similar to human.

Zebrafish possess 71.4% of the genes found in human and 82% of these are disease causing.



Zebrafish embryos used as models for human disease

Eg:

Teratogens eg: thalidomide

[Vertebrate embryos as tools for anti-angiogenic drug screening and function.](#)

Beedie SL, Diamond AJ, Fraga LR, Figg WD, Vargesson N. *Reprod Toxicol.* 2017 Jun;70:49-59.

Alcohol [Fetal alcohol spectrum disorders: Zebrafish in the analysis of the milder and more prevalent form of the disease.](#)

Seguin D, Gerlai R. *Behav Brain Res.* 2018 Oct 15;352:125-132.

Parkinson's disease [The developing utility of zebrafish models of neurological and neuropsychiatric disorders: A critical review.](#)

Fontana BD, Mezzomo NJ, Kalueff AV, Rosemberg DB.

Exp Neurol. 2018 Jan;299(Pt A):157-171. doi: 10.1016/j.expneurol.2017.10.004.

Obesity and diabetes [Zebrafish as a Model for Obesity and Diabetes.](#)

Zang L, Maddison LA, Chen W. *Front Cell Dev Biol.* 2018 Aug 20;6:91. doi: 10.3389/fcell.2018.00091.

Renal disease [Genetic Renal Diseases: The Emerging Role of Zebrafish Models.](#)

Elmonem MA, Berlingerio SP, van den Heuvel LP, de Witte PA, Lowe M, Levtchenko EN. *Cells.* 2018 Sep 1;7(9).

Eye diseases [Animal Models of Diabetic Retinopathy.](#)

Olivares AM, Althoff K, Chen GF, Wu S, Morrisson MA, DeAngelis MM, Haider N.

Curr Diab Rep. 2017 Aug 24;17(10):93. doi: 10.1007/s11892-017-0913-0.

Zebrafish are becoming increasingly useful for biomedical research, as models of human disease as well as to test effects of drugs to gain some insight into the potential actions of the drugs.

Indeed our research screening thalidomide analogs for versions that retain anti-inflammatory actions, but are not teratogenic – has identified 11 potential compounds (now patented) which are now being screened in multiple assays to determine clinical potential.

Furthermore, the UK NC3Rs agency recently advertised a new Grant scheme to encourage mammalian users to move into zebrafish research (see next slide).

Please see below an exciting opportunity to fund research on non-mammalian species provided by the **National Centre for the 3Rs**

Do you use non-mammalian model organisms in your research?

Are you a rodent user keen to explore how other whole organism models could benefit your research?

Are you interested in funding for a new research collaboration?

To encourage greater use of non-mammalian model organisms for 3Rs purposes, the NC3Rs has a highlight notice across all of its response mode funding schemes. Focusing on establishing collaborations between rodent and non-mammalian model organism users, the highlight notice will be launched at an NC3Rs workshop

Applications are invited using any of the following non-mammalian model organisms:

Nematodes e.g. *C. elegans*

Zebrafish embryos and larvae (prior to protection under the Animals (Scientific Procedures) Act)

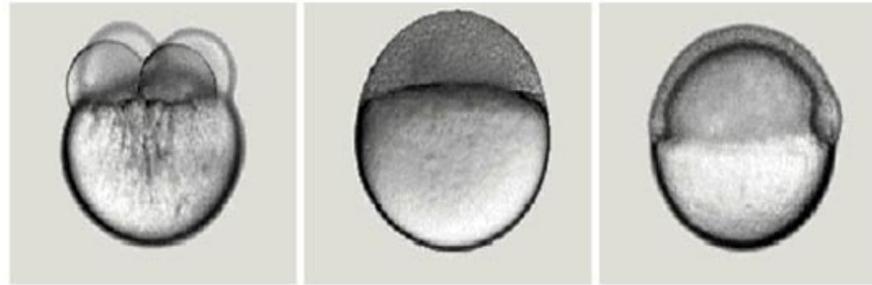
Drosophila

Galleria mellonella

Any area of biomedical research is in remit, but all applications must include a rodent user paired with a non-mammalian model organism user.

Closing date for registration is **21 August 2018**. More information on the [NC3Rs website](#).

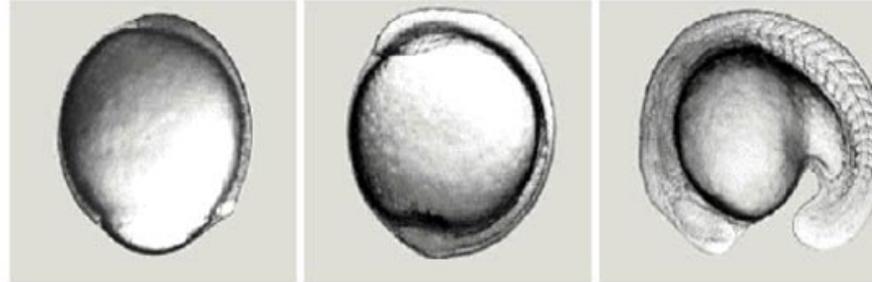
Developmental stages of Zebrafish development



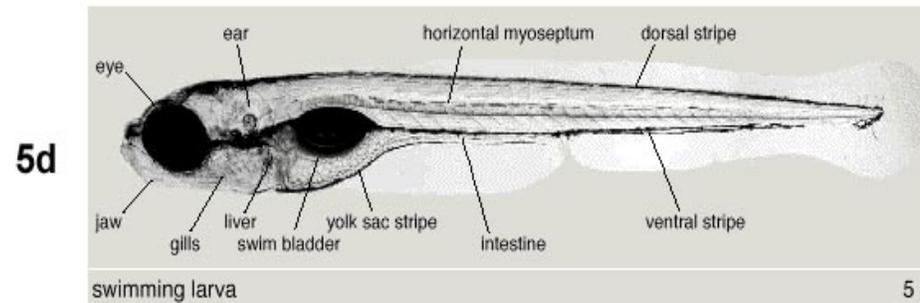
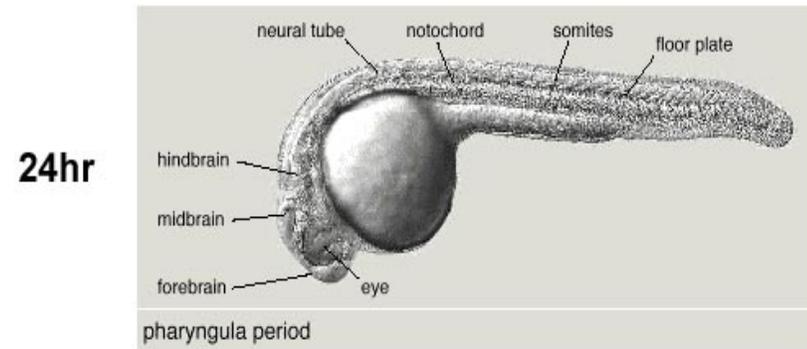
4 cell (1hr)

1K cell (4hr)

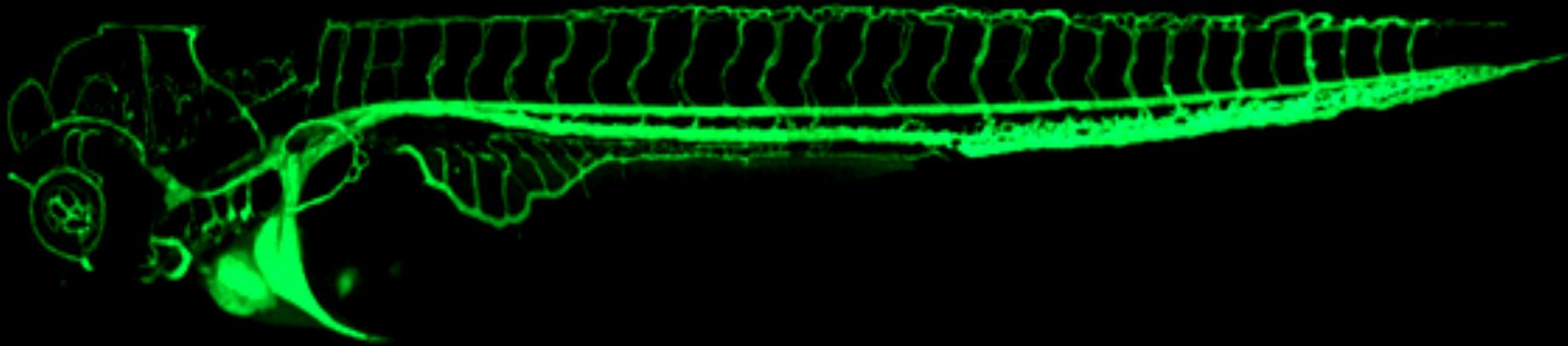
Shield (6hr)



80% epiboly (8hr) 1 somite (10hr) 19 somites (18hr)



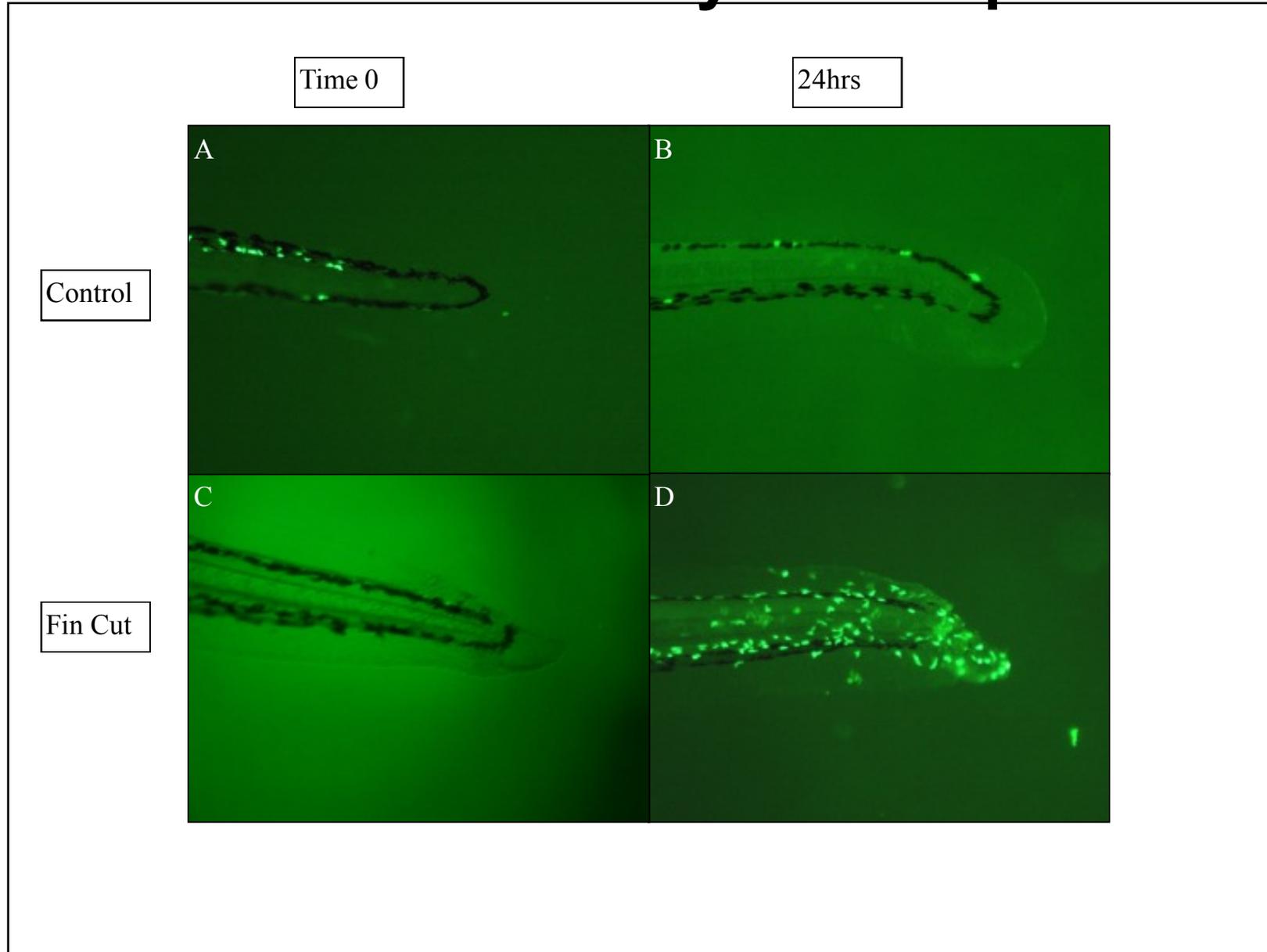
Vasculature of 2.5 day old Zebrafish embryo



EGFP expression in all endothelial cells under control of Fli1 promoter.

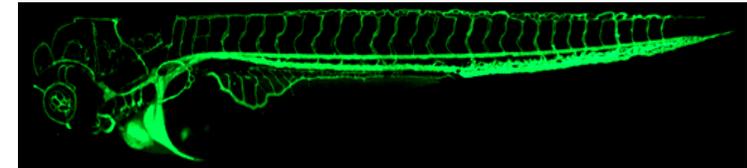
Lawson et al., 2002

Induction of neutrophils following tail fin cut in MPO:GFP embryos at 3dpf.



Screen of Thalidomide Analogs using zebrafish embryo models

- 89 compounds screened.
- Novel compounds:
 - 13 anti-angiogenic only
 - 11 anti-inflammatory only
- Lead compounds for further screening in higher species and human cell lines and ascertain molecular targets.
- This screening method reduces the number of large animals required for initial large scale drug screening and gives us an indication of what they might do in human's.
- Patents have been obtained for the analogs.



Overview

My lab is interested in understanding how drugs act on embryos, how some drugs cause damage and how they do that, and can we make safe forms of drugs that retain the benefits but not the side effects.

For more information see the following articles online:

<https://theconversation.com/is-primodos-the-forgotten-thalidomide-50673>

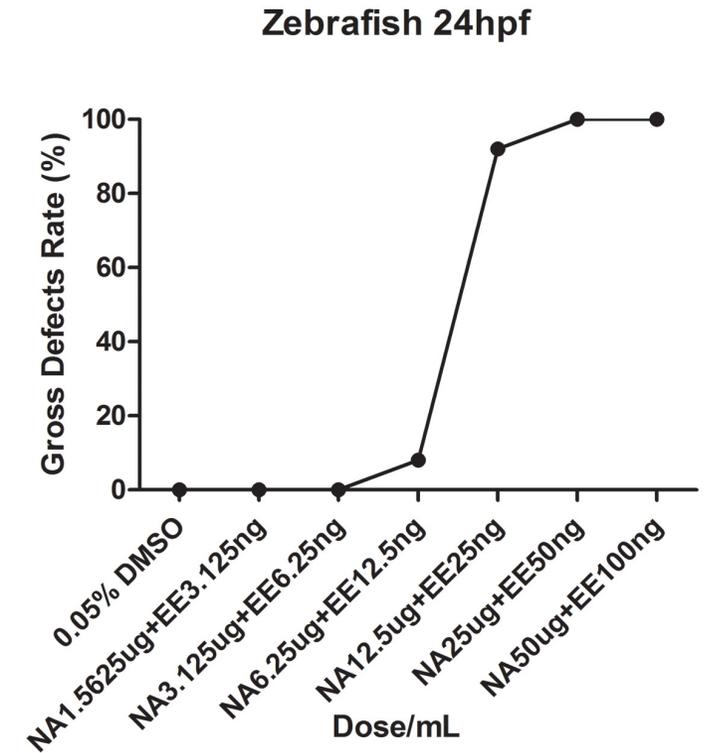
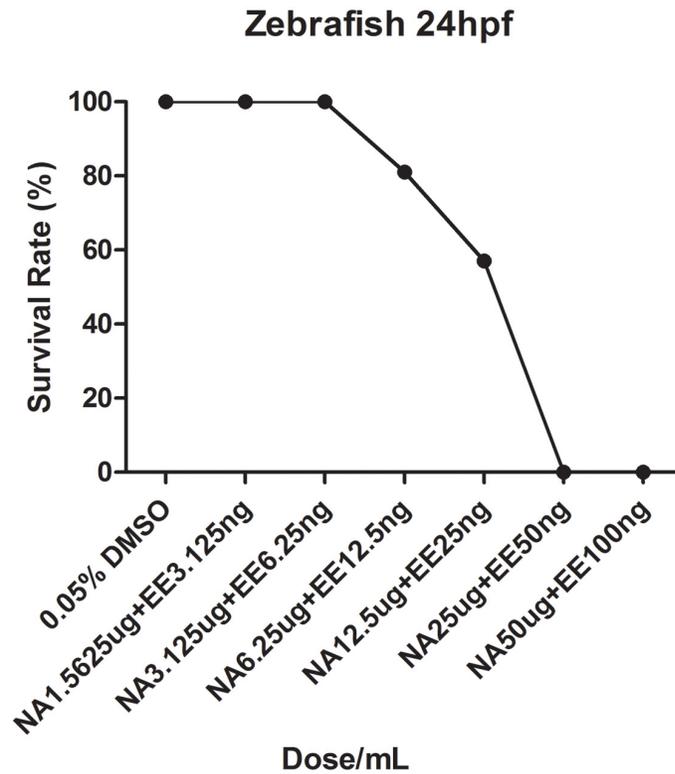
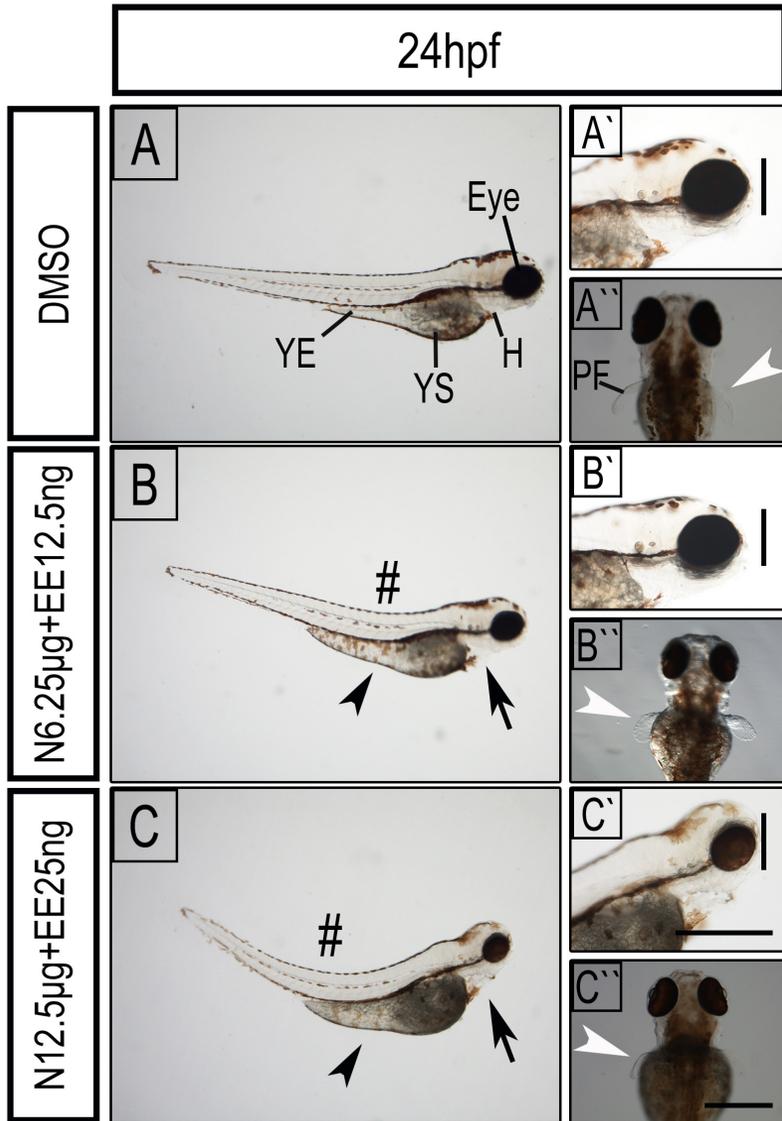
<https://theconversation.com/thalidomide-the-drug-with-a-dark-side-but-an-enigmatic-future-50330>

Dosing

For the Zebrafish embryo, we use 24 well plates and apply doses of the mixture in volumes of 500ul into wells holding 5-10 embryos. Doses are applied in a NA:EE 500:1 ratio to match the ratio of compounds in Primodos given to humans

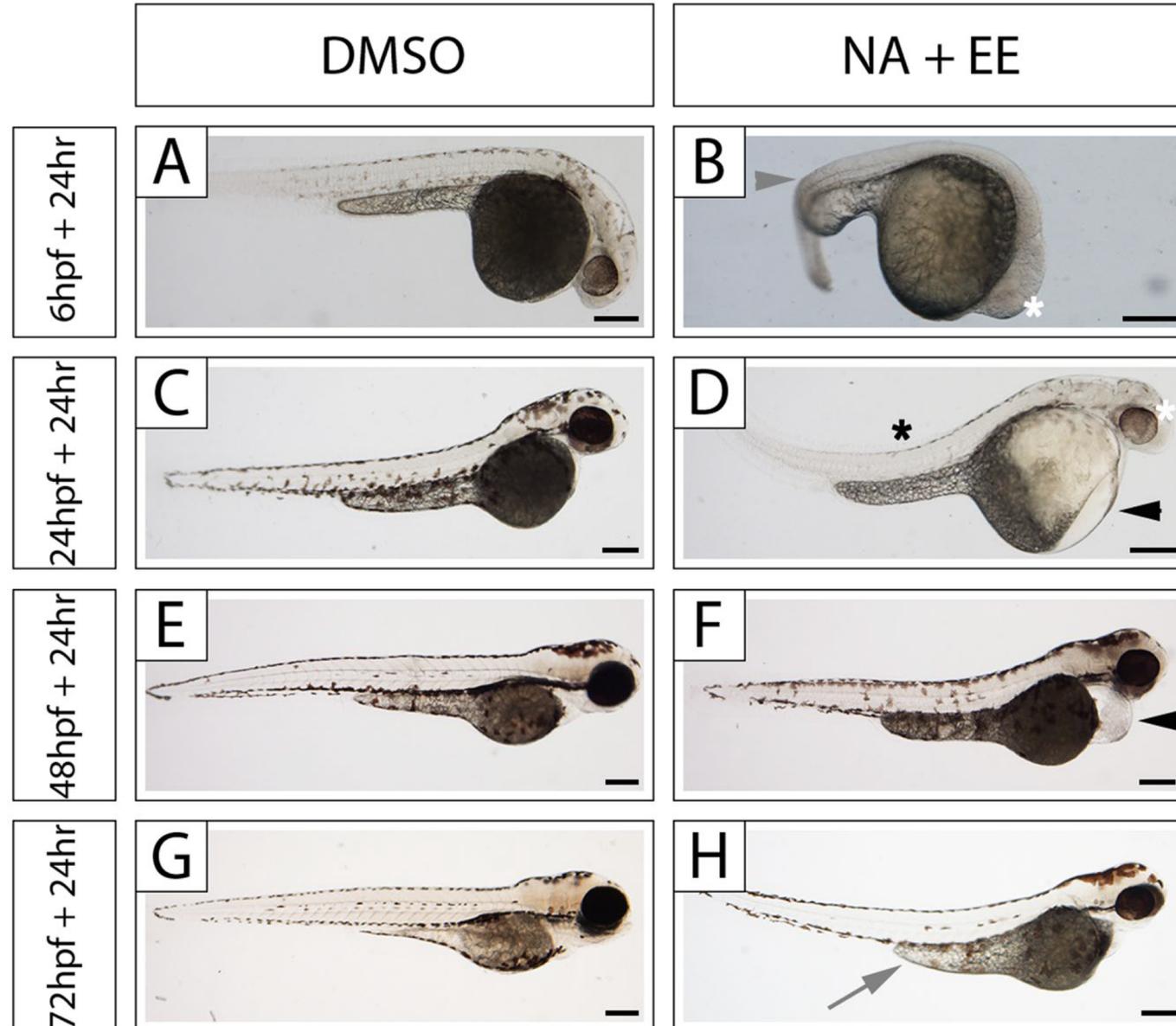
Typically larger doses of compounds are used in animal experiments – due to method of exposure, differences in uptake and metabolism, differences in bioavailability etc; we also found a dose response action of the drug.

NOR/EE-mixture induces damage in Zebrafish embryos

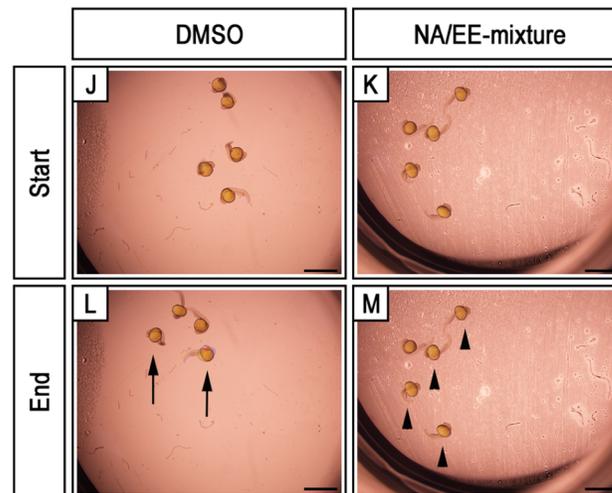
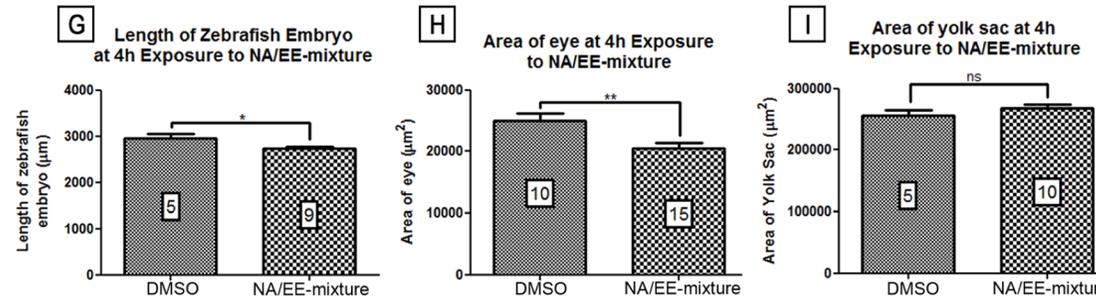
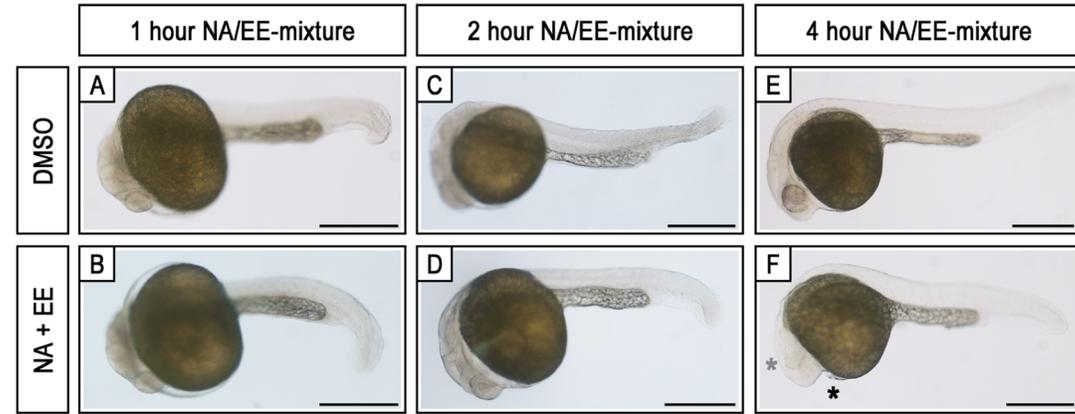


72hr after exposure at 24hpf

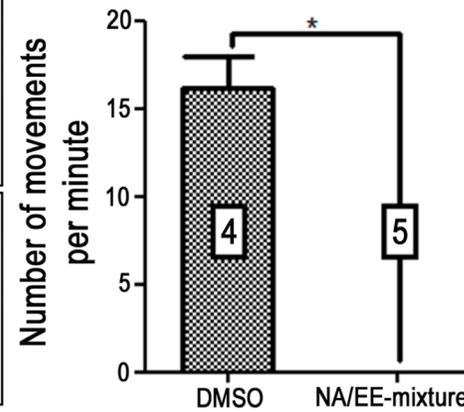
NOR/EE-mixture induces damage in Zebrafish embryos in a stage-sensitive manner.

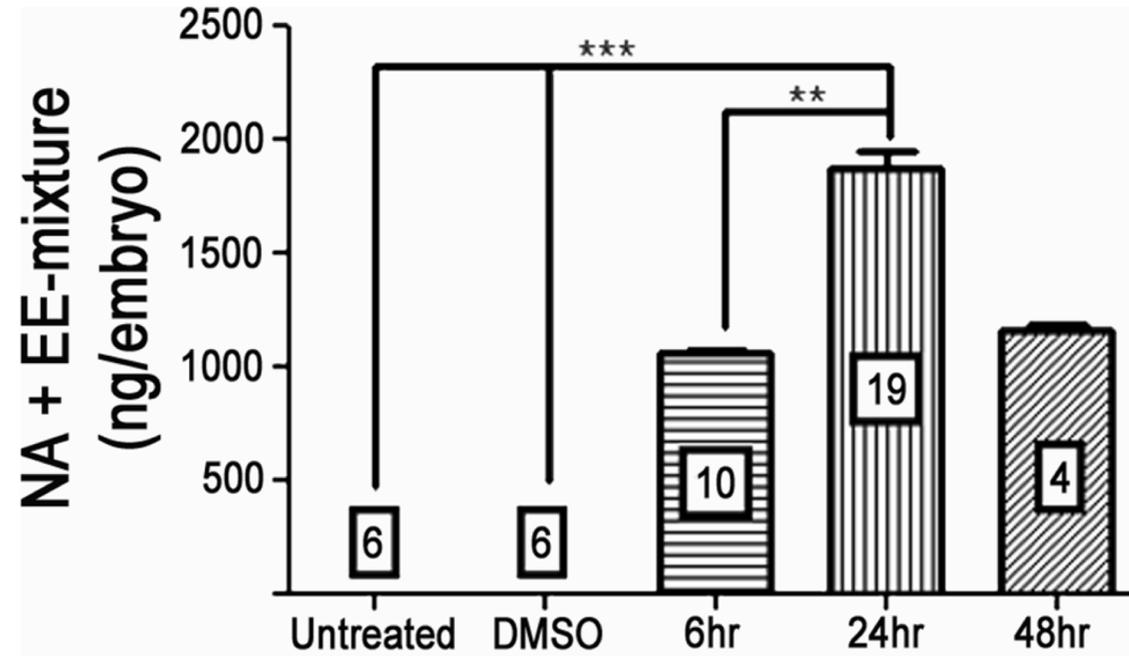


Morphological changes seen within 4hrs; though movement effects seen from 1hr



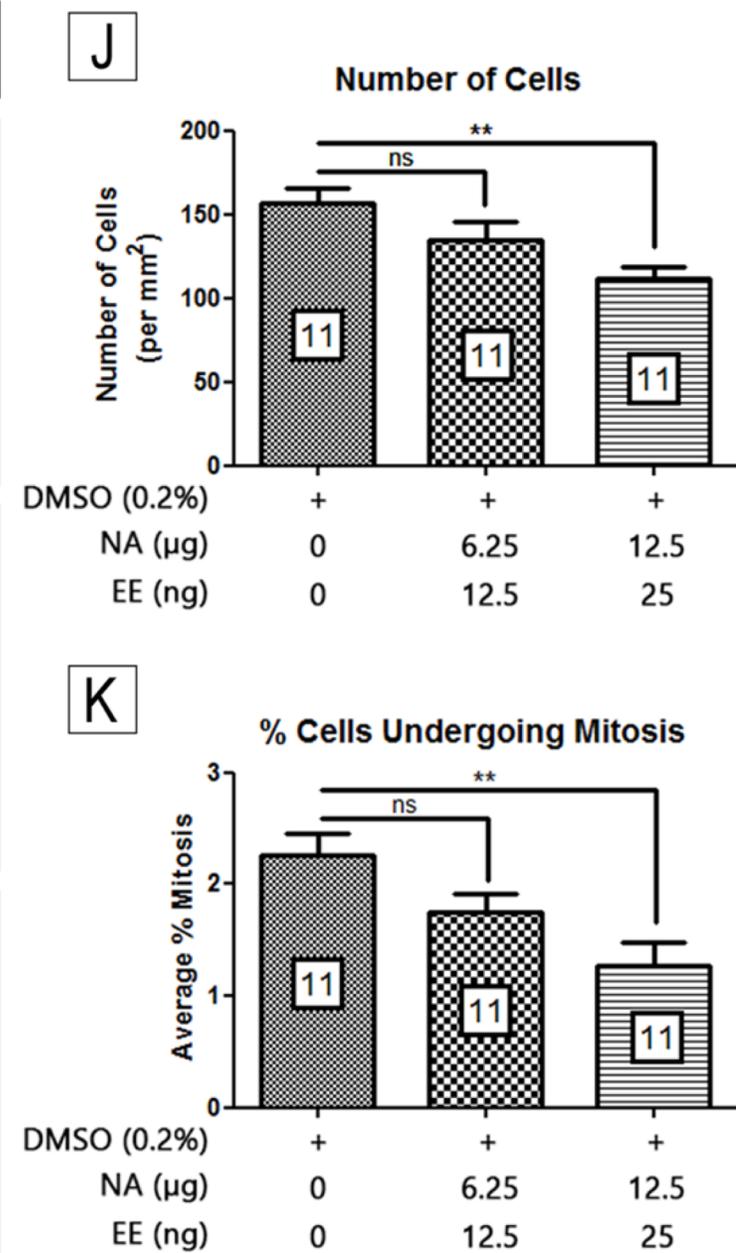
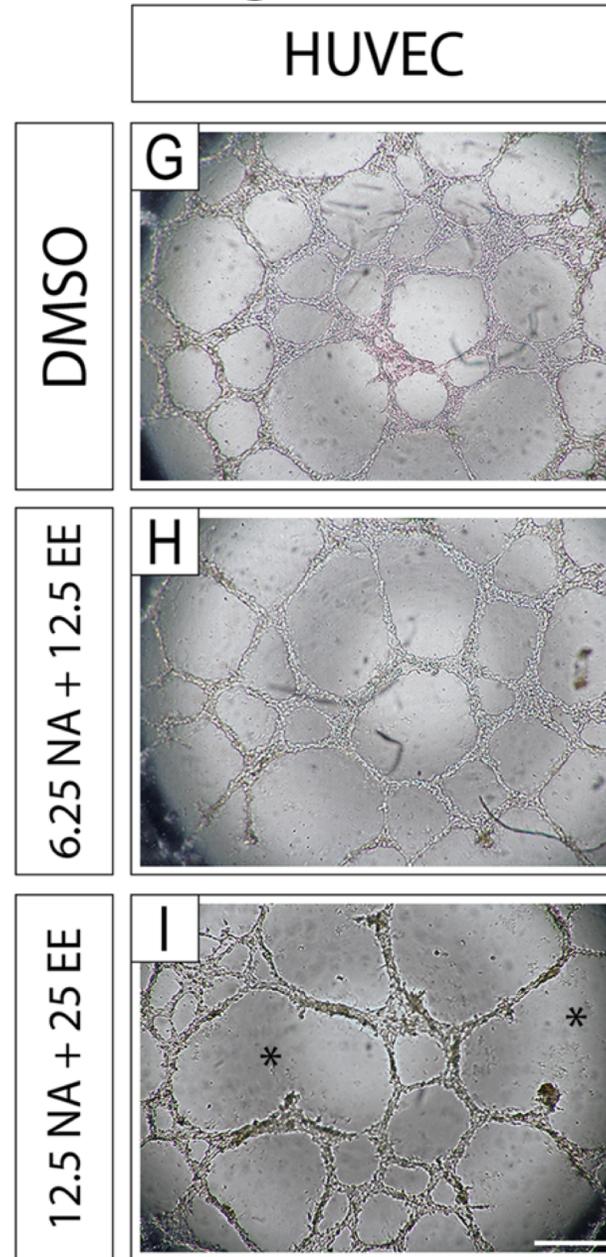
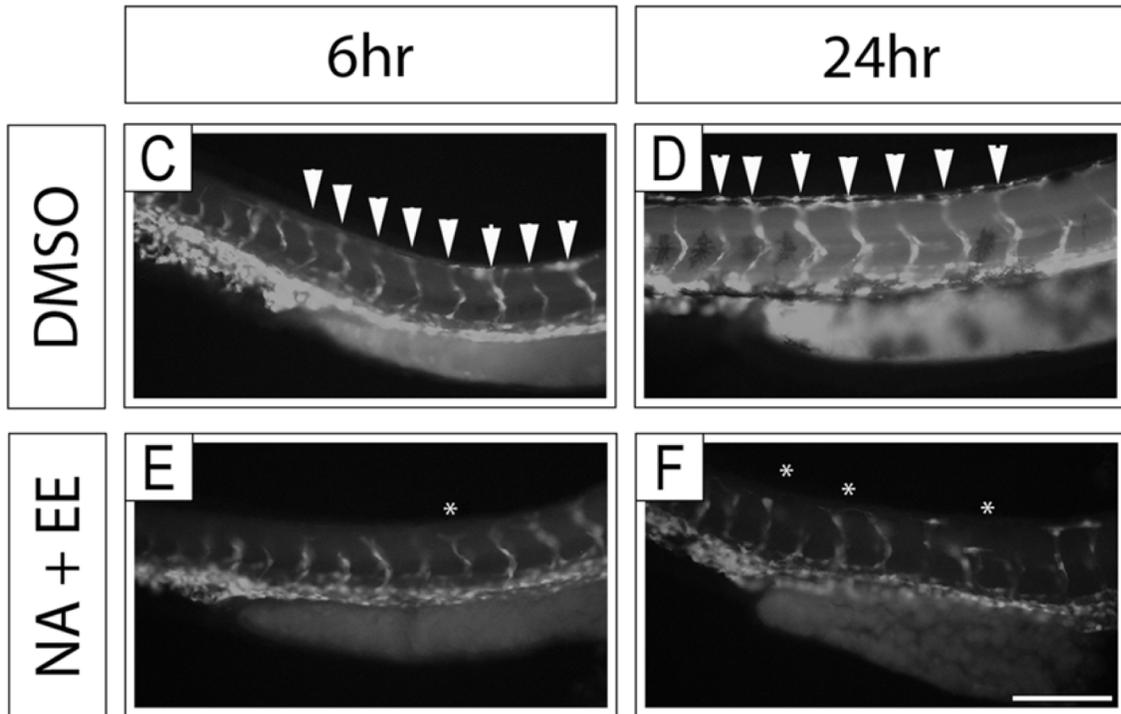
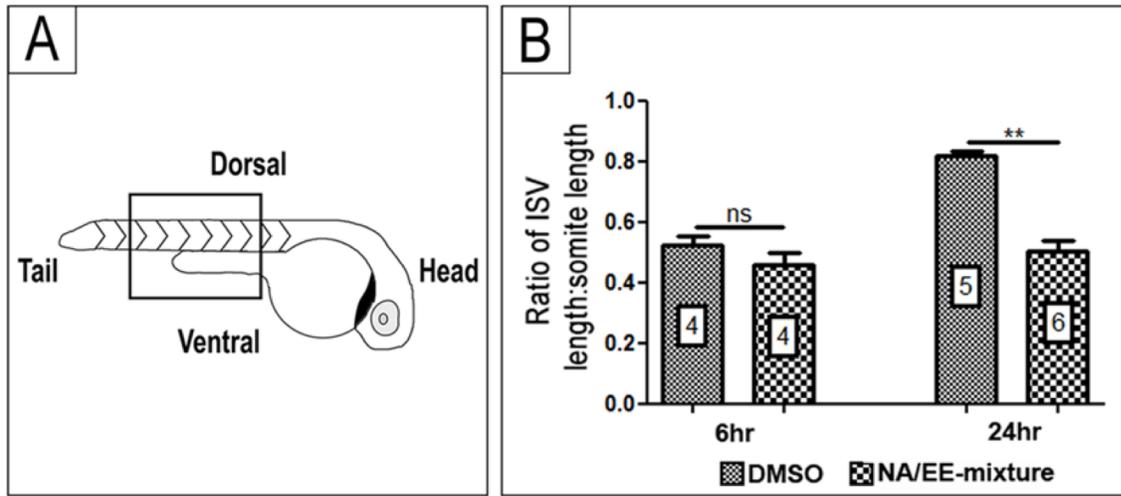
N Movement analysis at 1hr NA/EE-mixture exposure



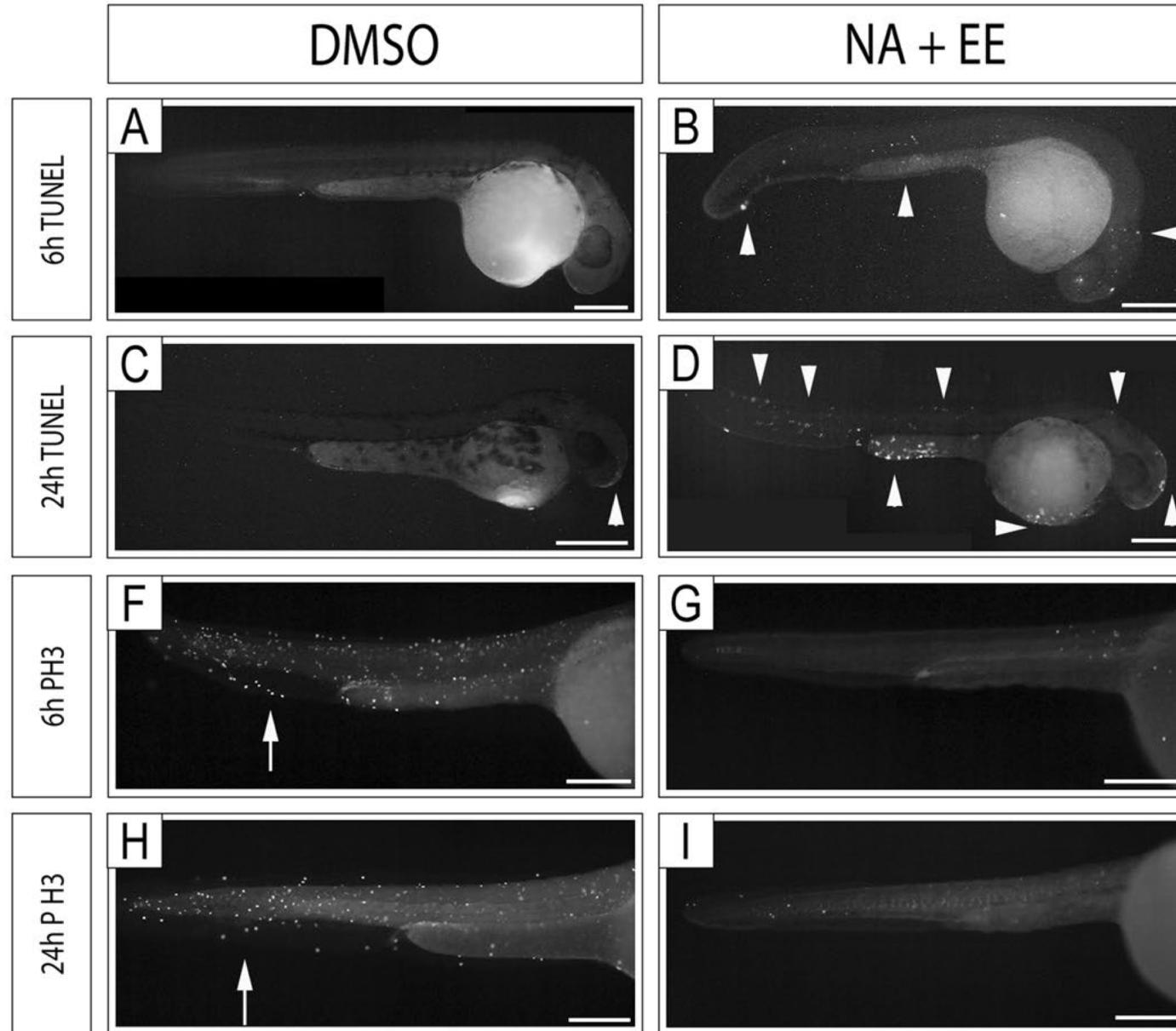


	6hr	24hr	48hr
Untreated	0 ± 0 (n=6)	0 ± 0 (n=10)	0 ± 0 (n=5)
DMSO	0 ± 0 (n=5)	0 ± 0 (n=11)	0 ± 0 (n=5)
NA/EE-mixture	1077 ± 19.05 (n=5)	1872 ± 71.65 (n=19)	1164 ± 22.57 (n=4)

Rapid blood vessel changes following NOR/EE-mixture exposure

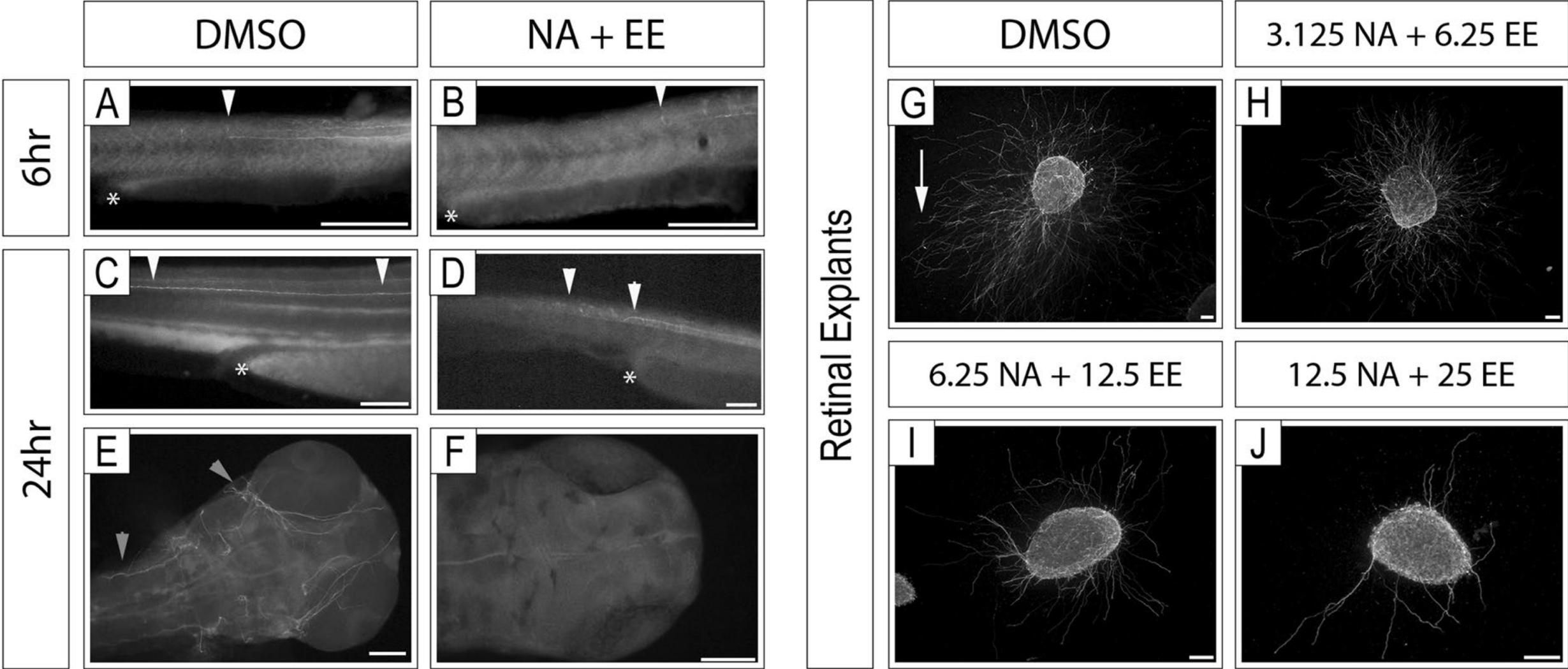


Cell Death is increased and Cell Proliferation decreased in Zebrafish Embryos Treated with NA/EE-mixture



In vivo and in vitro neuro-inhibitory effects of NA/EE-mixture exposure

NA/EE-mixture effects on nerve outgrowth and patterning.



Summary of Brown et al., 2018 study

- Identified a range of doses that causes damage to zebrafish embryos.
- Confirmed the compound acts in a time-sensitive manner.
- Damage to zebrafish embryo tissues occurs from around 4hr. Movement issues from 1hr.
- We observe fin and eye developmental changes as well as pericardial oedema, yolk sac changes, bent spines and reduced movement. We also observe changes to cell death and cell proliferation. We also observe damage to nerves and inhibition of nerve in *in-vitro* cultures which might explain the reduced ability to move.
- We observe changes to blood vessels which might explain fin and eye damage (and spine damage).
- Demonstrate effects in zebrafish embryo, human cell lines and mouse tissue cultures (via direct exposure)
- From Mass spectroscopy studies identified a fraction of the compound in the water surrounding the embryo gets into the embryo, builds up for 24hr before levels then reduce.
- Further work needed to determine the mechanisms by which the mixture is causing changes to embryo development – multiple species would be beneficial.
- Indicates this compound can harm zebrafish embryos – more work required to determine if can do similar things to mammalian embryos

Embryo's partially recover 72hr after being placed in fresh water following 24hr treatment

We have evidence that 24hr old embryos treated with NA/EE for 24hrs and then placed in fresh water – can partially recover– but damage to eyes, eats and fins is still evident at 96hrs.

We also show that levels of NA are significantly reduced in the embryo within 24hrs of being placed in water (obtained via Mass Spectroscopy).

What are we doing now?

1. Zebrafish molecular analyses*

Starting some RNA sequencing of treated embryos to determine molecular changes.

2. Mouse embryo organ/tissue culture

Started with gonads, will also try kidney, limb, lung, liver.

3. Human fetal organ culture*

Idea is to culture organs and tissues from human fetal material and test effect of compounds

4. Chicken embryo*

Application is by dripping the dose of the compound over the embryo.

*partially dependent on obtaining grant funding (which I am trying to obtain) as such experiments are costly.

Limitations of Brown et al., 2018 study

-Dose

Very high compared with physiological relevant dose seen in humans. Thus, not possible to correlate to human directly.

Many factors affect the dose given to zebrafish – these include:

- receptor specificity,
- tissue diffusion of compound,
- kinetics and bioavailability differences,
- plasma binding protein differences,
- human synthetic hormone vs zebrafish embryo,
- difference in method of exposure,
- metabolic or hydrolytic byproducts,
- placental effects.

There are reports that supraphysiological doses of compounds are required in zebrafish to obtain a physiologically relevant dose (eg from a paper studying estradiol uptake in zebrafish embryos – Souder and Gorelick, 2017 – Quantification of estradiol uptake in zebrafish embryos and larvae. *Tox. Sci.* 158: 465-474)

Zebrafish used as a model to study thalidomide embryopathy and doses used are in the range of 400-800uM to induce damage – these doses are higher than used in human but reciprocate the condition in zebrafish and is used to model the condition (Ito et al., 2010).

Rodents were used in 1960's-70's to study NA/EE and found high doses were embryo-lethal – though didn't assess if embryo was damaged and that is why died – assumptions were it was toxic.

-Mechanism

We see effects on nerves, blood vessels, tissue morphogenesis and gene expression profiles. Direct exposure to zebrafish embryo, human cells and mouse tissue cultures results in effects to the tissues. Suggests direct exposure to drug can cause problems.

Will be important to study effects in mammalian models and also investigate any effect on placenta to determine if vessels/nerves could be affected compromising placental function.

Vascular inhibition/insufficiency has been linked to birth defect causation for many years – indeed, its one of the main mechanisms of action that thalidomide is proposed to act through. (Vargesson and Hootnick, 2017. Reproductive Toxicology 70: 21-29).

We have carried out some preliminary gene expression analysis on genes involved in nervous system development and also a muscle marker – to see if this could explain lack of movement of embryos and note changes in expression profiles throughout development.

Neurological effects could be a separate mechanism in addition to vascular effects. Several synthetic progestins disrupt the glial cell specific-brain aromatase expression in developing zebrafish (includes Norethindrone and Ethisterone). Cano-Nicolau et al., 2016. Toxicology and Applied Pharmacology 305 (2016) 12–21

Further work is required to establish the mechanistic basis of NA/EE actions.

Levels of Norethisterone peak rapidly and then take time to be cleared/removed from maternal plasma in human pregnancy

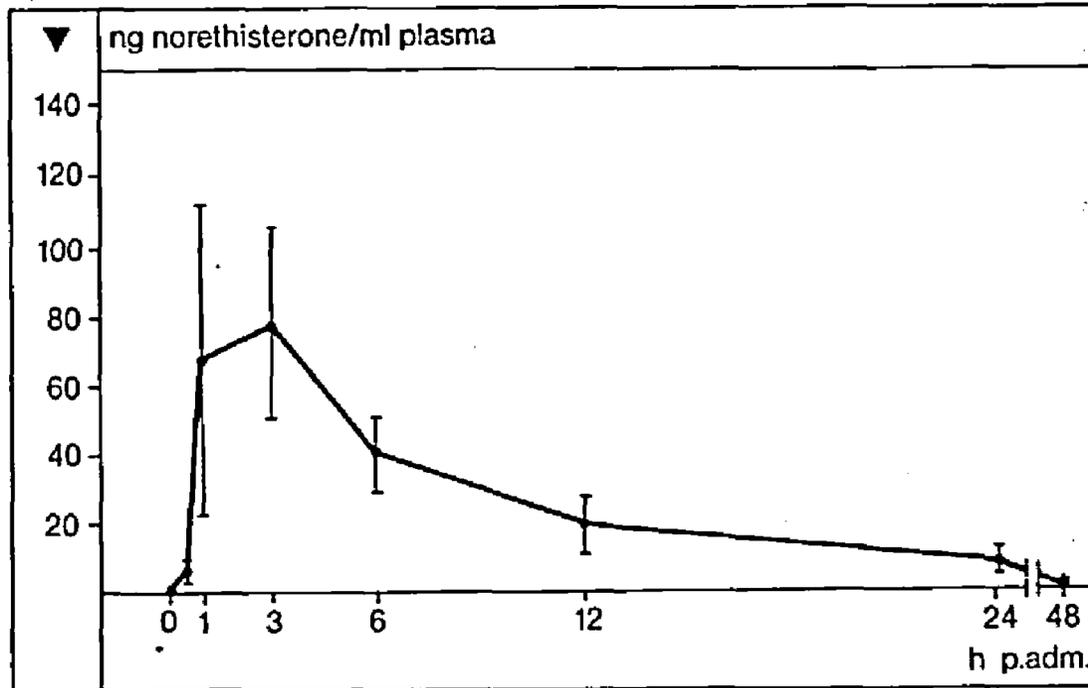


Fig. 2. Peripheral plasma levels of norethisterone during 2 days in early pregnancy of 11 women treated with a single oral dose of 20 mg of norethisterone acetate and 0.04 mg of ethinylestradiol ($\bar{X} \pm SD$).

Study indicated 17/35 drug exposed pregnancies had haemorrhaging.

This is **not** normal. Whether exposed fetuses exhibited defects is not discussed.

Haemorrhaging and vascular disruption is known to cause birth defect.

[Malformations attributed to the process of vascular disruption.](#)

Holmes LB, Westgate MN, Nasri H, Toufaily MH. Birth Defects Res. 2018 Jan;110(2):98-107.

[Hemorrhage in development of the face.](#)

Poswillo D.

Birth Defects Orig Artic Ser. 1975;11(7):61-81.

-Specificity

As we conclude, further work is needed in mammalian species to confirm the effects seen in this study. Zebrafish develop rapidly and outside the body, thus are not placental. However, they do not have metabolic function for some time (like human embryos) thus build up of compounds/chemicals in the embryo/yolk sac is possible.

Zebrafish do have 70% of the genes, humans have and are being used to model human disease, so are clearly relevant – but further work in a range of animal models is needed to confirm and extend these findings as well as drug toxicity/actions.

Summary

As it stands, our study indicates the potential for this compound to harm Zebrafish embryos – whether it harms mammalian embryos or not (which are placental) remains to be determined. But Zebrafish embryos are good indicators to alert or raise concerns for follow up with further studies.

We are following up our zebrafish studies – looking for the mechanism by which the drug acts and using mammalian and human tissues to also help understand the compound action.

Acknowledgements

Dr Lucas Fraga

Dr Samantha Brown

Prof Lynda Erskine

Amanda Berg

Gary Cameron

Elizabeth Stewart

Zoe Finlayson

Funding

**Undergraduate Lab-based project allowances
from University of Aberdeen**



**UNIVERSITY OF
ABERDEEN**