COMMISSION ON HUMAN MEDICINES

ZEBRAFISH AD HOC WORKING GROUP

Minutes of the meeting held on 5th October 2018 at 12:00 p.m. in the Round Room, 10th floor, 10 South Colonnade, Canary Wharf, London, E14 4PU

Participants Present

Members

Professor A Boobis (Chair)
Professor L Levy
*1 Professor A Piersma
Professor M Placzek
Professor A Smith
Professor S Wilson

Visiting Expert

Professor N Vargesson

Observers

Mrs M Lyon
Dr Sonia Macleod

Expert provided written comments/written input

Dr L T.M. van der Ven

Apologies

Mr N Dobrik

Secretariat

Ms E Agca

MHRA Legal

Mr Z Fargher

Professional Staff of MHRA Present

Supporting Specific Items

Dr J Clements – *VRMM, scientific assessor*
Dr R Hawkes – *VRMM, scientific assessor*
Mrs S Morgan – *VRMM, Group Manager*
Dr J Raine – *VRMM, Divisional Director*

Observers

Mr C Attrill – MHRA *Head of Patient, Public and Stakeholder engagement*
Mrs L Loughlin – *Head of Science Strategy*

VRMM - Vigilance and Risk Management of Medicines Division

*1 *Joined meeting via teleconference*

1. Apologies and Announcements

1.1 The Chair reminded those present that the papers and proceedings are confidential and should not be disclosed and that all mobile phones must be switched off.

1.2 The Chair informed those present that the proceedings will be recorded for minute taking purposes. The recording will be destroyed once the minutes of the meeting have been agreed.

1.3 All those present introduced themselves.

The Chair clarified that the meeting participation is divided into the following categories:

Chair & Members – are invited to attend for the whole meeting and are able to contribute to the conclusions and recommendations of the group.

Visiting Expert – is invited to present their work to the group and for the discussion of this work and will not contribute for the conclusions and recommendations.

Observers – are invited to attend for the whole meeting, except for the conclusions and recommendations, where they will be asked to leave the meeting. In carrying out her role as a member of The Independent Medicines and Medical Devices Safety Review Team, if she wishes, Dr Macleod may stay for the discussion of conclusions and recommendations. Dr MacLeod indicated her wish to remain for these discussions.

1.4 The Chair reminded those present to declare any personal interests (e.g. shares, lecture fees, consultancy, travel/accommodation costs or other direct remuneration) in the following associated companies:

Successors of the companies who originally marketed HPTs:

- Alinter Group
- Bayer plc
- GlaxoSmithKline UK
- Marshall's Pharmaceuticals Ltd
- Merck, Sharpe and Dohme Ltd
- Pfizer
- Piramal Healthcare Ltd
- Sanofi

The companies who originally marketed HPTs:

- Roussel Laboratories
- Parke Davis
- Wallace Manufacturing Chemists Ltd
- Schering
- Organon Laboratories
- Nicholas Laboratories Ltd
- Duncan Flockhart and Company Ltd.

The Chair reminded participants to declare the nature of any involvement they may have had with HPTs (e.g. reviews of these products, public commentary on their safety).

The register of interests declared by participants was made available in advance of the meeting. The Chair informed those present that legal advice on the declared interests had been sought and that the declared interests had not been deemed to debar any participation. There were no concerns raised. No further interests were declared.

1.5 The Chair informed members and observers that Professor Piersma was joining the meeting via teleconference, as he was unable to attend in person due to the cancellation of his flight. Apologies for the day were received from Mr Dobrik.

2. Matters Arising

2.1 The chair advised those present that should they be contacted by the media as a result of this meeting, they should refer the queries to the MHRA Press Office.

The details are:

- email newscentre@mhra.gov.uk
- phone 020 3080 7651 (08:30 - 17:00) or 07770 446 189 (17:00 - 08:30)

Press office details were circulated to all participants.

3. Terms of Reference

The following Terms of Reference of the Group had been endorsed by the Commission on Human Medicines, to consider:

- The suitability of the zebrafish model for evaluating the effects of norethisterone and ethinylestradiol in human pregnancy;
- The robustness of the study; and
- Any clinical implications

And to advise the CHM

3.1 The meeting attendees noted the Terms of Reference and opportunity to comment on them was provided. No comments were noted.

4. Key points for consideration

4.1 The MHRA presented the key points from their assessment report for the Expert Group to consider. As background, the MHRA first outlined relevant regulatory guidance. The MHRA concluded by indicating areas that the Group might wish to focus on, i.e.

- Advantages and potential limitations of zebrafish as a model organism for studying norethisterone and ethinylestradiol in human pregnancy outcomes
- NET/EE receptor pharmacology and pharmacodynamics in zebrafish and humans
- Route of administration, timing and duration of zebrafish embryo and clinical exposures
- The accumulation levels of NETA achieved in zebrafish embryos and levels determined in mammalian embryos

The Group thanked MHRA for the clear and balanced summary. Mrs Lyon raised a comment that three published papers referenced in the assessment report were from Schering-sponsored studies and two of these were highlighted in the discussion.

5. Professor Vargesson's Presentation

5.1 The Chair made Professor Vargesson aware that the meeting would be voice recorded to assist the generation of the minutes and this will be deleted once the minutes have been agreed. No objections by Professor Vargesson were noted.

5.2 Professor Vargesson presented highlights from the published paper *Brown et al., 2018*, additional preliminary data, and future planned studies.

5.3 The Group queried the approach to dose selection for the *Brown et al., 2018,* experiments. Professor Vargesson clarified that dose selection was based on selecting a dose range where effects were observed.

5.4 The Group considered that the array of effects observed could be due to mechanisms other than via "classical" receptors, to which Professor Vargesson agreed.

5.5 The Group highlighted the importance of determining the actions of the individual components of the NETA/EE mixture to elucidate the mechanisms involved. Professor Vargesson responded that only preliminary data was available.

5.6 The Group raised methodological questions on blinding to phenotypic observations, technical replicates and reproducibility for the zebrafish development experiments. Professor Vargesson explained that blinding was challenging due to lack of embryo movement following treatment but highlighted that two PhD students had individually performed the experiments. Professor Vargesson also confirmed the numbers of technical and study replicates.

5.7 The Group asked whether the effects of the mixture on zebrafish embryo movement were reversible. Professor Vargesson commented that movement effects, but not developmental effects, were partially recovered by 96 hours in drug containing medium. Professor Vargesson further explained that additional data had shown that movement effects were reversible when embryos were transferred to water following drug exposure, with reversibility observed from 24 hours with almost full recovery observed at 96 hours.

5.8 The Group commented on whether the developmental effects were in fact due to developmental delay, considering that the embryo morphology images provided in *Brown et al. 2018* suggest the embryos are not developing at the normal rate, thus making phenotype comparisons between treatment and control groups challenging. Professor Vargesson agreed that developmental delay is possible but damage to some of the tissues would suggest not all of the effects could be due to this.

5.9 The Group asked whether the concentration ranges used were appropriate to separate the reversible movement effects from the developmental effects. Professor Vargesson commented that further studies were planned to address this point.

5.10 The Group thanked Professor Vargesson for his helpful presentation and responses to their questions.

6. Discussion of the data presented by Professor Vargesson

6.1 The Group acknowledged the strengths and limitations of the study and agreed that the findings were interesting, but there are a number of unknowns that need to be resolved before the implications of the findings for possible risks to humans can be determined.

6.2 The Group noted the difficulties of interpreting the findings in the absence of a mechanism and consequently the clinical relevance of the pleiotropic responses in zebrafish was unknown.

6.3 The Group highlighted the importance of testing the individual mixture components separately and considered the possibility of using antagonist or CRISPR based approaches in order to elucidate the underlying mechanisms involved. Professor Vargesson had performed some work on this, suggesting that the effects were due primarily to NETA.

6.4 The Group noted the high drug concentrations at which thresholds for the effects were observed.

6.5 The Group noted the wide range of effects in many different tissues and cell types, and thus effects were unlikely to be mediated through primary pharmacology alone. The Group considered it likely that if similar exposures were used with other animal tissues, similar phenotypes would be observed as these high concentrations appear to be damaging to cells and cell viability.

6.6 The Group noted the importance of the fact that there is a defined concentration threshold at which no effects are observed. The Group acknowledged that knowledge of subtle effects could be further refined by looking at lower concentrations to obtain a further detailed dose-response analysis.

6.7 The Group determined that accumulation levels within the zebrafish embryo were very high and potentially equated to as much as 1% of the total embryo wet weight.

6.8 The Group noted the very high accumulation of norethisterone acetate in zebrafish embryos compared to mammalian fetuses. The Group acknowledged this could be due to a range of factors and speculated that differences in protein binding, and subsequent availability of free drug, between the zebrafish media and mammalian maternal plasma may play a role.

6.9 The Group noted that for Primodos, which was dosed just once on two consecutive days, accumulation would not be the relevant consideration. The maximum concentration in the embryo would be more pertinent.

6.10 The Group determined that even with conservative estimates the zebrafish embryo exposures were very likely to be several orders of magnitude higher than the likely exposure in humans.

6.11 The Group noted that the zebrafish is a useful model for exploring the qualitative effects of chemicals in general on development, screening and product design but interpretation of the results would depend on the class of compound. Regarding NETA/EE specifically, there is a lack of information on the pharmacology, mechanism/s, pharmacokinetics and exposure-response relationship to determine the specificity and subsequently the relevance of effects to humans.

7. Post-discussion announcements

7.1 The Chair reminded those present that in line with the participation definitions stated in the invitation letters, the Visiting Expert and Observers are not permitted to contribute to conclusions and recommendations. The following participants left the meeting at this point:

Visiting Expert
Professor N Vargesson

Observer
Mrs Marie Lyon

Dr Macleod stayed for the discussion of conclusions and recommendations in carrying out her role as an observer from The Independent Medicines and Medical Devices Safety Review Team.

8. Conclusions and advice for the Commission on Human Medicines -

The Group returned to the Terms of Reference and reached the following conclusions which were agreed unanimously.

8.1 The suitability of the zebrafish model for evaluating the effects of norethisterone and ethinylestradiol in human pregnancy:

8.1.1 The Group considered that zebrafish can be a useful model system for studying developmental toxicity but there are currently limitations such that translation of the observed effects to human pregnancy outcomes is not possible. Although developmental processes are highly conserved between fish and humans there are molecular and physiological differences that can affect the specificity of a response. The model has been used for identifying potential mechanisms at the molecular target level and generating information for key events in an adverse outcome pathway rather than direct extrapolation to humans. The model in general can be used to complement, rather than provide an alternative to, established regulatory mammalian developmental toxicity assays.

8.1.2 The Group concluded that the zebrafish model can provide information on qualitative effects of chemicals in general, however, for NETA/EE, there is a lack of information on the pharmacology, pharmacokinetics and mechanisms that makes interpreting the relevance of the observed findings for humans challenging.

8.2 The robustness of the study:

8.2.1 The Group acknowledged that the *Brown et al., 2018* study was generally well conducted and that the limitations of the study were recognised by the authors. The Group concluded that the observed developmental effects were general and occurred in a range of different organ systems. It was noted that effects occurred on a steep concentration gradient and that lethality overlapped with developmental effects. The group determined that the effects were pleiotropic in nature and that further investigation could reveal additional effects. There are no mechanistic explanations for the observed effects, many of which are most likely non-classical receptor mediated.

8.3 Any clinical implications:

8.3.1 The Group concluded that knowledge gaps existed 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. Developmental effects occurred at concentrations in the zebrafish embryo that were several orders of magnitude higher than would occur following clinical doses. Consequently, the Group considered that the *Brown et al., 2018* study should be considered with the existing evidence as part of the overall weight of evidence and concluded that the study does not raise any new safety concerns for products in clinical use containing norethisterone acetate and ethinylestradiol.

9. Any other Business

No additional issues were raised.

10. Meeting close

The meeting closed at 2:58pm

VRMM
10 October 2018

Alan R Boobis OBE

18/10/18