Considerations of maternal toxicity in classification

George Daston
Overview

• Maternal toxicity: how much is too much?
  • -what do guidelines tell us?
  • What is the scientific consensus?
  • Using weight of evidence
• The importance of adjunct studies
  • Isolated whole embryo experiments
  • Mode of action
• Case studies
• Areas of uncertainty
Maternal toxicity in developmental toxicity studies

• Body weight gain

• Food consumption
  • Body weight and food consumption are measured repeatedly, and the measurements are readily comparable across labs

• Mortality

• Clinical/ cageside observations
  • These are done by all labs, but reporting criteria and level of detail is unlikely to be comparable across labs
Maternal toxicity: interpretation per CLP guidance

• Development can be influenced by toxic effects in the mother
  • May be non-specific, related to stress or disturbance of homeostasis
  • May be a specific, maternally-mediated mechanism

• Ideally, clear evidence of a reproductive effect in the absence of systemic toxicity
  • But discounting developmental effects can only be done when the role of maternal toxicity in adverse development is shown to be causally related

• Use expert judgment and a weight of evidence approach
Maternal toxicity: how much is too much?

- Mortality: 10% or greater
  - EU, OECD, US and other guidelines all agree on this bright line

- Significant clinical signs
  - Not a lot of specific guidance
    - Coma, ataxia, hyperactivity, labored breathing are given as examples

- Decreased maternal weight gain
  - No bright lines (except in dev. neurotox guideline, for which >10% is excessive)
  - greater than a 10-20% reduction, per HESI consensus workshops (less than 10% is not a concern, greater than 20% is excessive)
Considerations on decreased food consumption

• Pfizer studies on developmental effects of feed restriction in rats and rabbits
  • As a means of understanding the consequences of controlling for the anorectic effects of weight-loss drugs
  • outcome: fetal weight effects and variations but no malformations

• Misinterpretation:
  • Because decreased body weight/ feed consumption does not by itself cause much in the way of adverse developmental outcome, then toxic effects that decrease weight and feed consumption cannot adversely affect development
  • This makes the erroneous assumption that the decreased maternal weight is in the causal chain from exposure to developmental toxicity, rather than a separate manifestation of the same underlying mechanism of toxicity
Weight-of-evidence

• Effects at more than one dose
  • If so, are they consistent?
    • Same effects with a dose-related increase in prevalence and severity

• Individual animal data
  • Are some dams more affected than others?
  • Is the developmental toxicity restricted to those litters?

• Historical control data

• Multiple developmental toxicity studies
  • Reproducibility of findings
    • Qualitative: same kinds of effects?
    • Quantitative: same rate of response?
  • A more complete dose-response curve, if doses are staggered
Weight of evidence

• Data from repeated-dose studies
  • Almost always have a more robust assessment of adult toxicity
    • Clinical chemistry
    • histopathology
  • Longer dosing period
    • May help reveal effects
    • But is not matched to the duration of a Segment 2 study
• Animals not pregnant
  • Physiological effects are probably not qualitatively different, but may be different in magnitude
Mode of action

• Some examples of maternally-mediated developmental toxicity
  • Maternal anemia or hypoxia
    • Hemolytic anemia in rabbits from diflunisal
    • Blood loss
    • Diminished cardiac function
    • Uterine vasoconstriction
  • Maternal acid-base balance
  • Functional zinc deficiency
    • Induction of metallothionein in maternal liver
  • Maternal intermediary metabolism
example: alpha-hederin

<table>
<thead>
<tr>
<th>Dose (umol/kg)</th>
<th>0</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal weight (g)</td>
<td>3.49</td>
<td>3.11</td>
<td>3.01</td>
</tr>
<tr>
<td>Malformations</td>
<td>3/242</td>
<td>6/175</td>
<td>9/154</td>
</tr>
<tr>
<td>MT (ug/g/ liver)</td>
<td>3</td>
<td>59</td>
<td>49</td>
</tr>
<tr>
<td>Liver Zn (nmol/g)</td>
<td>350</td>
<td>575</td>
<td>550</td>
</tr>
<tr>
<td>Plasma Zn (nmol/g)</td>
<td>14.5</td>
<td>11</td>
<td>9.5</td>
</tr>
</tbody>
</table>

- Transfer of Zn to embryos also compromised
- WEC: no effect of direct addition of alpha-hederin
- Increasing dietary zinc ameliorates the effects
Example: diflunisal

<table>
<thead>
<tr>
<th>Dose (mg/kg/d)</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal weight (g)</td>
<td>38.7</td>
<td>37.9</td>
<td>35.4</td>
<td>35.1</td>
</tr>
<tr>
<td>Malformed fetuses</td>
<td>9/99</td>
<td>6/97</td>
<td>16/107</td>
<td>12/29</td>
</tr>
<tr>
<td>Maternal H’crit</td>
<td>37</td>
<td>33</td>
<td>24</td>
<td>20</td>
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</table>

Treatment only on GD 5

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>0</th>
<th>180</th>
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<tbody>
<tr>
<td>Fetal weight</td>
<td>34.9</td>
<td>34.3</td>
</tr>
<tr>
<td>Malformed fetuses</td>
<td>6/177</td>
<td>6/21</td>
</tr>
</tbody>
</table>
Use of WEC to interpret in vivo rodent results

• Removes embryo from maternal influences
  • Can determine if chemical has direct effects on the embryo
  • Important to consider possible metabolism
  • Important to consider pharmacokinetics in selecting concentrations to test
    • Top concentration should mimic or exceed Cmax or 24-hour AUC from the in vivo maternally toxic concentration
WEC applications: examples

• Direct embryotoxicity vs. secondary effects
  • Identifying chemicals that decrease circulating zinc in vivo by MT induction, but have no direct embryotoxicity
  • Agents that affect cardiovascular function
  • others

• Identifying active metabolite
  • Research on ethylene glycol identifying glycolic acid as the active teratogen, and not metabolic acidosis
Case study: cyanamide

Rat Seg. 2 study

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>0</th>
<th>5</th>
<th>15</th>
<th>45</th>
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</thead>
<tbody>
<tr>
<td>Mat. Wt. gain, GD6-16</td>
<td>50</td>
<td>41</td>
<td>32</td>
<td>6</td>
</tr>
<tr>
<td>Mat. Wt. gain, GD6-20</td>
<td>106</td>
<td>94</td>
<td>81</td>
<td>50</td>
</tr>
<tr>
<td>Fetal weight</td>
<td>3.26</td>
<td>3.19</td>
<td>3.13</td>
<td>2.84</td>
</tr>
<tr>
<td>Post-imp. Loss (%)</td>
<td>3.6</td>
<td>2.3</td>
<td>3.7</td>
<td>7.3</td>
</tr>
<tr>
<td>Diaphragmatic hernia</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
</tbody>
</table>

Reviewer conclusions: maternal NOAEL is <5 mkd because of 20% decrease in weight gain over the dosing period. Developmental NOAEL is 5 mkd based on fetal weight effects at the mid-dose.
Weight of evidence

- Effect seen in rat but not rabbit
- Historical control
  - Not provided, but rate of diaphragmatic hernia is probably higher than background
- Mode of action
  - AlDH inhibition leading to functional retinoic acid deficiency
    - Note: submitter argued that only the low Km forms were inhibited
  - Diaphragmatic hernia is one of several reported manifestations of functional RA deficiency
    - Note: submitter argued that because other manifestations were not observed, this was not evidence of a syndrome
- Dossier submitter: category 2, because malformations were only observed in the presence of severe maternal toxicity
Case study: cycloxidim

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>0</th>
<th>100</th>
<th>200</th>
<th>400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mat. Wt. gain, GD6-15</td>
<td>43</td>
<td>42</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Fetal weight</td>
<td>3.85</td>
<td>3.82</td>
<td>3.74</td>
<td>3.65</td>
</tr>
<tr>
<td>Skeletal variations (%)</td>
<td>6</td>
<td>8</td>
<td>6</td>
<td>34</td>
</tr>
</tbody>
</table>

- Increase in BUN, creatinine at 400 mg/kg/day
- No effect in WEC up to concentrations equivalent to the Cmax at 400 mkd
- Postnatal study: persistence of dumbbell-shaped ossification sites at PND 21
Weight of evidence

• Multiple studies, all with basically the same conclusion
• Increases in BUN and creatinine were presented but not mentioned in the evaluation of maternal toxicity
• WEC results presented but not discussed in the decision on classification
Classification

• Dossier submitter
  • No classification proposed

• RAC discussion
  • concern by one member that persistence of the dumbbell ossification pattern was a malformation

• Additional public comment period
  • Two comments received, with divergent opinions about whether the dumbbell vertebrae were malformations
  • Lots of discussion in the final commentary about when to consider dumbbell vertebrate malformations, based on good science from teratologists

• Final opinion: category 2, based on the conclusion that the maternal toxicity was not severe enough to produce the observed effects
Conclusions

• Bright lines on what constitutes excessive maternal toxicity are available for some, but not all, manifestations
• Mechanistic information that connects the specific maternal mode of action with developmental toxicity is important
• WEC is a useful, but perhaps underutilized tool
• Weight of evidence assessment is crucial for good decision making
  • We should provide more guidance on how to make it consistent and robust