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

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

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

Example: diflunisal

Dose (mg/kg/d)	0	20	40	60
Fetal weight (g)	38.7	37.9	35.4	35.1
Malformed fetuses	9/99	6/97	16/107	12/29
Maternal H'crit	37	33	24	20

Treatment only on GD 5

Dose (mg/kg)	0	180
Fetal weight	34.9	34.3
Malformed fetuses	6/177	6/21

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

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

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

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

- 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