Developmental Toxicity (Teratology)

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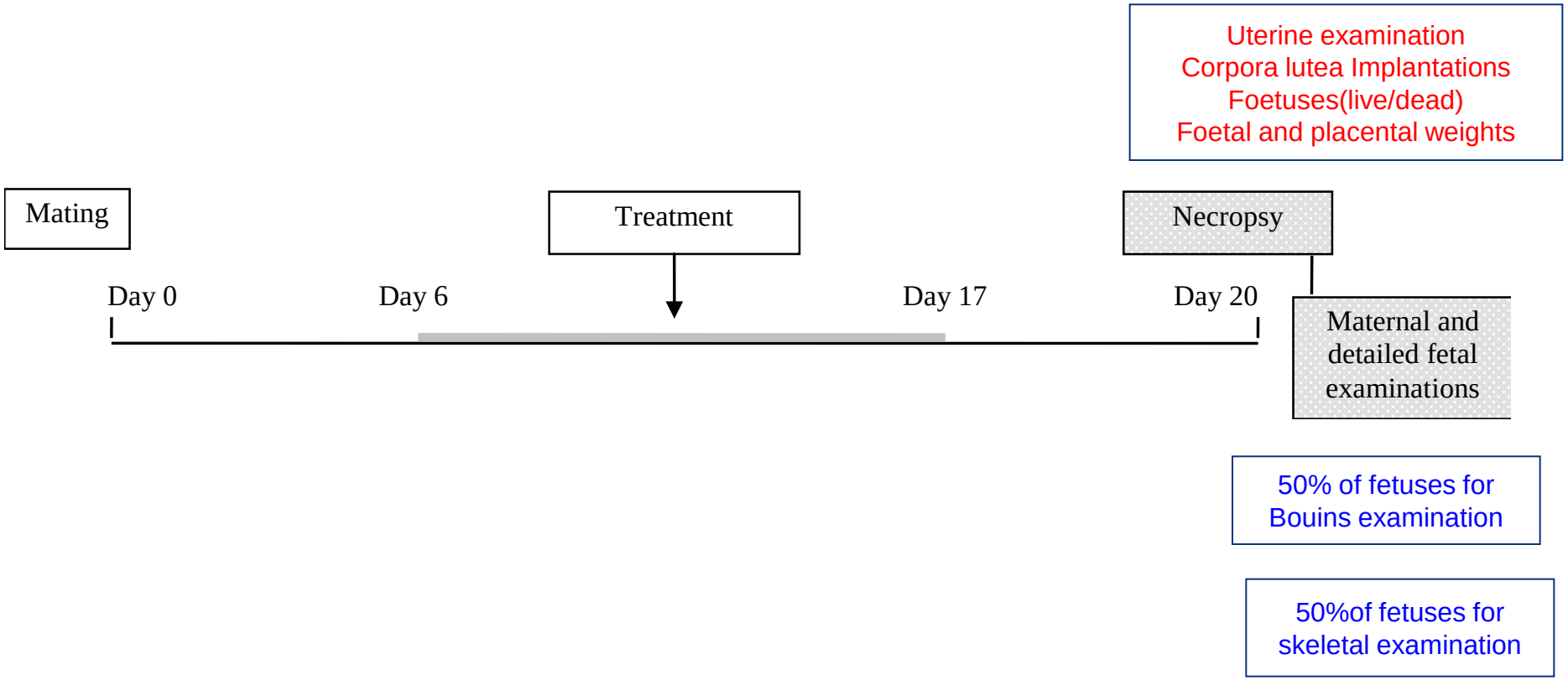
Study Design



Parameters Assessed

In-Life

- Bodyweight - Daily
- Food Consumption - Intervals
- Clinical Observations

Post-Mortem

- Pregnancy status
- Organ weights - ovaries, pituitary
- Numbers and distribution of :
  - Corpora lutea
  - Implantations
  - Early and late deaths
  - Live implants

Post mortem evaluation

4 groups of 22 female rats

- 80 pregnant females
- 1100 – 1200 fetuses
- +/- 550 skeletal examinations
- +/- 550 visceral examinations

4 groups of 20 female rabbits

- 72 pregnant females
- 650 – 700 fetuses
- all fetuses skeletally and viscerally screened

Container	Solution	Time	Temp. (° C)	Vacuum	
1	3% KOH	4u30’	30	ja	maceration and clearing
2	1% KOH, 0.005% Alizarine RedS	8u00’	30	ja	staining
3	0.1% KOH, 10% glycerine	8u00’	30	ja	destaining soft tissue
4	50% glycerine	4u00’	30	ja	destaining soft tissue
5	60% glycerine	4u00’	30	ja	destaining soft tissue
6	70% glycerine	4u00’	30	ja	destaining soft tissue
7	80% glycerine	4u00’	30	ja	destaining soft tissue
8	gedemineraliseerd water	10’	-	-	destaining soft tissue

Fetal Pathology

Classification of fetal observations:

- Malformation: rare and/or probably lethal major abnormality (e.g. exencephaly)

- Minor abnormality: minor deviation from “normal” found relatively frequently (e.g. dilated lateral ventricles)

- Variant: structures which occur relatively frequent in the control population (e.g. extra pair of ribs)

New techniques

Medical imaging techniques are being explored by the industry
- micro CT-scanning
- MRI

Advantages
- non-invasive technique
- longitudinal studies possible
- automated image analysis possible

Problems
- expensive equipment
- scanning time
- amount of data collected (CT: 0.5GB/fetus 600 GB/study)

Feasibility study

Study set-up

- dosing pregnant female rats with a known toxic compound
- part of the study: necropsy on day 21
- part of the study: natural delivery around day 22
- scan the pups on day 1, 7, 21, 100

Objective

- compare images of CT with “golden standard”
- evaluate the new technique for mechanistic studies

MATERNAL TOXICITY

janssen
PHARMACEUTICAL COMPANIES
OF Johnson & Johnson

What is Maternal Toxicity?

Classical Definition

"An effect in pregnant/lactating females that differs in nature or degree from that of a non-pregnant/lactating female receiving the same dosage."

Reality

This is a general term such that :

Any observed toxicity in the parental female is generically termed maternal toxicity

Regulatory requirements - EPA / OECD

‘The dose levels should be spaced such that the highest dose induces toxicity but not more than 10% death in the parental animals.’

Regulatory requirements - JMAFF

‘ the highest dose level should be chosen with the aim to induce toxicity but not death or severe suffering. In case of mortality this should not be more than approximately 10% in the parental animals’.

Regulatory requirements - ICH

These guidelines are much less proscriptive

factors limiting the highest dosage determined from repeat dose toxicity studies or preliminary repro studies could include :

Regulatory requirements - ICH

However, the guidelines for both the embryo-fetal and pre- and post-natal studies also state that one of the aims of the study is to assess

'enhanced toxicity relative to the that in non-pregnant females'

Regulatory requirements - EPA / OECD

The middle dose should produce minimal but observable toxicity and the lowest dose level will be a no effect level

'In a study which demonstrates an absence of toxic effects, further investigations to establish absorption and bioavailability of the test material should be considered'.

Regulatory requirements - ICH

- Reduction in bodyweight gain
- Increased bodyweight gain
- Specific target organ toxicity
- Haematology / clinical chemistry
- Exaggerated pharmacology
- Physico-chemical properties
- Kinetics

Prenatal toxicity is considered to be especially significant below the level of adult toxicity.

‘With pronounced signs of maternal toxicity in man no one would exclude the possibility of an induction of prenatal damage, and in such a situation adult toxicity becomes the limiting factor in risk assessment.’

Neubert D. Teratology 1992

Often it is difficult to distinguish between effects mediated through the parents versus direct interaction with the developmental processes. For example, developmental toxicity may be influenced by the effects of toxic agents on the maternal system when exposure occurs during pregnancy or lactation.

However, problems arise when "teratogenicity" is (falsely) considered to be a characteristic of maternal toxicity, since it is difficult to exclude that direct embryo-foetal toxicity is first evident at the same dose-level as that of maternal toxicity.

- **The minimum amount of information considered useful for evaluating maternal toxicity** includes morbidity or mortality, maternal body weight and body weight gain, clinical signs of toxicity, food and water consumption (especially if dosing is via food or water) and necropsy for gross evidence of organ toxicity.

End Result

We make do with clinical signs, body weight and/or food consumption as our main predictor for dose level selection

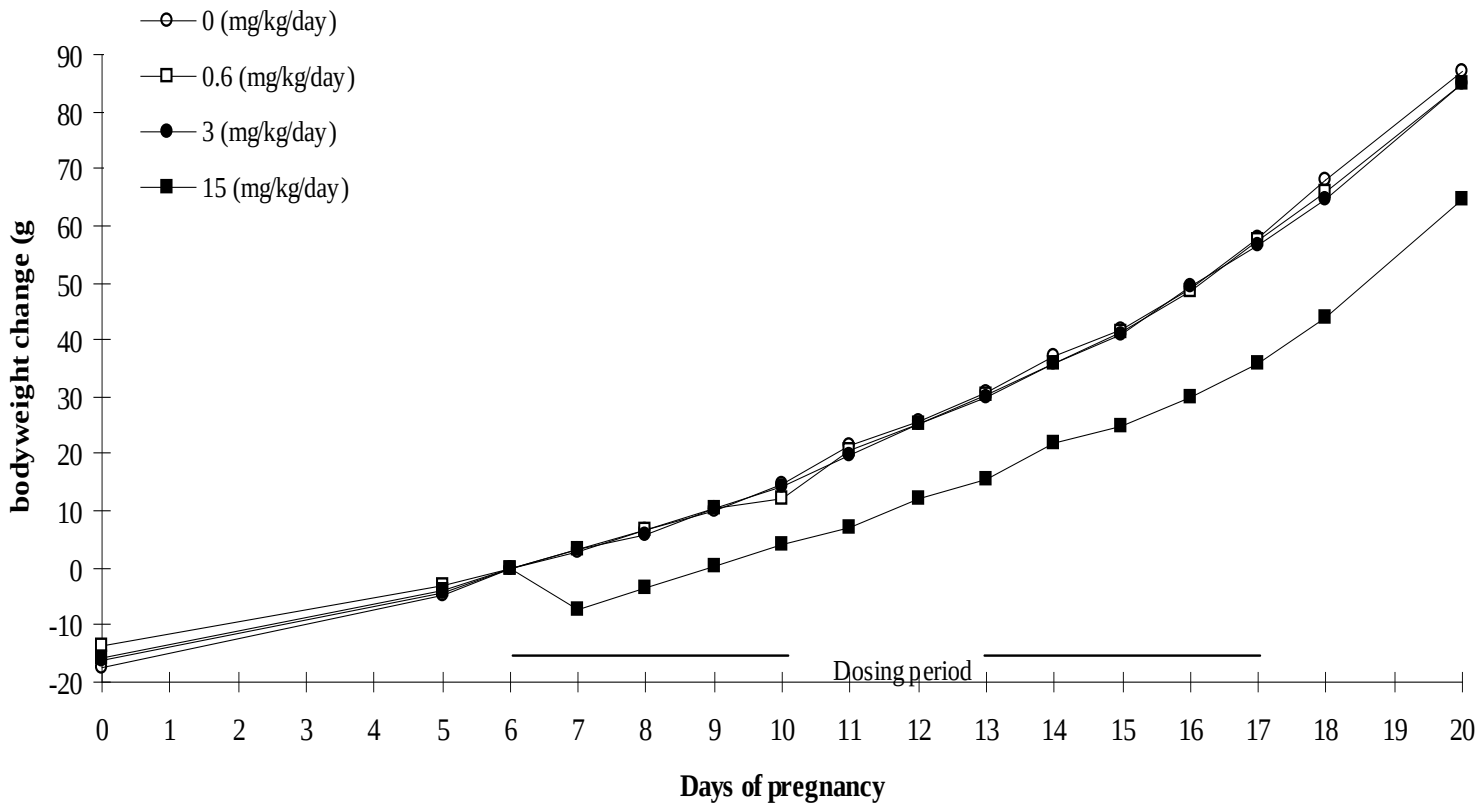
What do we end up trying to achieve ?

- Generally we are looking for a slight but clear indication of toxicity
- This is often represented as a 10% reduction in bodyweight gain over Controls (difficult in rabbits)
- A 10% reduction in food consumption over a proportion of the dosing period.

Bodyweight

Figure 1

Group mean maternal bodyweight change (g)



Food Consumption

Days of Pregnancy	Group 1	Group 2	Group 3	Group 4
5 to 6	19 ± 3	17 ± 3	17 ± 3	18 ± 3
6 to 9	20 ± 2	19 ± 2	18* ± 5	15** ± 2
9 to 12	22 ± 2	20 ± 2	21 ± 2	18** ± 2
12 to 17	23 ± 2	22 ± 2	21 ± 2	17** ± 2
17 to 20	23 ± 2	24 ± 6	22 ± 2	25* ± 7
6 to 17	22 ± 2	21 ± 2	20 ± 2	17** ± 2

It is not simply the slight reduction in weight or food consumption that induces abnormal pre-natal development, but the differences in metabolism and/or function resulting from these which are responsible for causing these events.

What can we expect

- Reduced foetal weight !?
- Reduced level of ossification !?
- Increased level of resorptions !?
- Increase in incidence of abnormalities/malformations !?

Foetal weight / ossification

- Would it be surprising if foetuses were lighter if maternal weight reduced !
- Foetuses with lower foetal weight generally have lower levels of ossification !
- Bodyweight is the best measure of pup development post-natally

Ossification parameters

Areas most obviously affected :

- Sternebrae
- Metacarpals/metatarsals
- Phalanges (Rabbit)
- Pelvis (Pubis)
- Epiphyses (Rabbit)
- Hyoid
- Cervical/Caudal vertebrae
- Skull - interparietal, Supra-occipital

Increased Embryo/Foetal Loss

Foetal loss can be induced simply by reduction in food intake with a consequential reduction in bodyweight

Matsuzawa et. al. Toxicology 22. (1981)

We must be careful, however, that any observed maternal toxicity is not in fact foetal toxicity.

If the test article causes foetal loss then the weight gain of the mother during pregnancy will be less due to the reduced total litter weight and the food consumption generally slightly lower. Therefore it would be easy to ascribe the foetal losses to the reduction maternal weight gain (maternal toxicity) rather than the other way round.

Abnormalities / Malformations / Variations

- Commonest - Supernumerary ribs

Not going to discuss - multitude of references available

Cleft palate (mouse)

Rib anomalies - fusions, wavy

Vertebral anomalies - fused, split

Sternebral anomalies - fused, misaligned

Major blood vessel variations (maternal hypotension)