Addendum to NAC-43 Meeting Summary.

Following the developmental toxicity discussion presented by Marcel van Raaij, George Woodall discussed recent work performed at U.S. EPA, NCEA in Research Triangle Park, NC (Addendum Attachment). Dr. Woodall's presentation focused on the use of developmental endpoints as points-of-departure for developing acute reference values. A comparison of repeated versus single exposure data was presented for two chemicals, ethylene oxide and butyl benzyl phthalate.

Addendum attachment

Developmental/Reproductive Toxicty: Comparison of Repeated versus Single Exposures for Two Model Compounds

Allen Davis, MSPH, and
George Woodall, PhD

Presented to NAC/AEGL 43rd Meeting
Rotterdam, Netherlands

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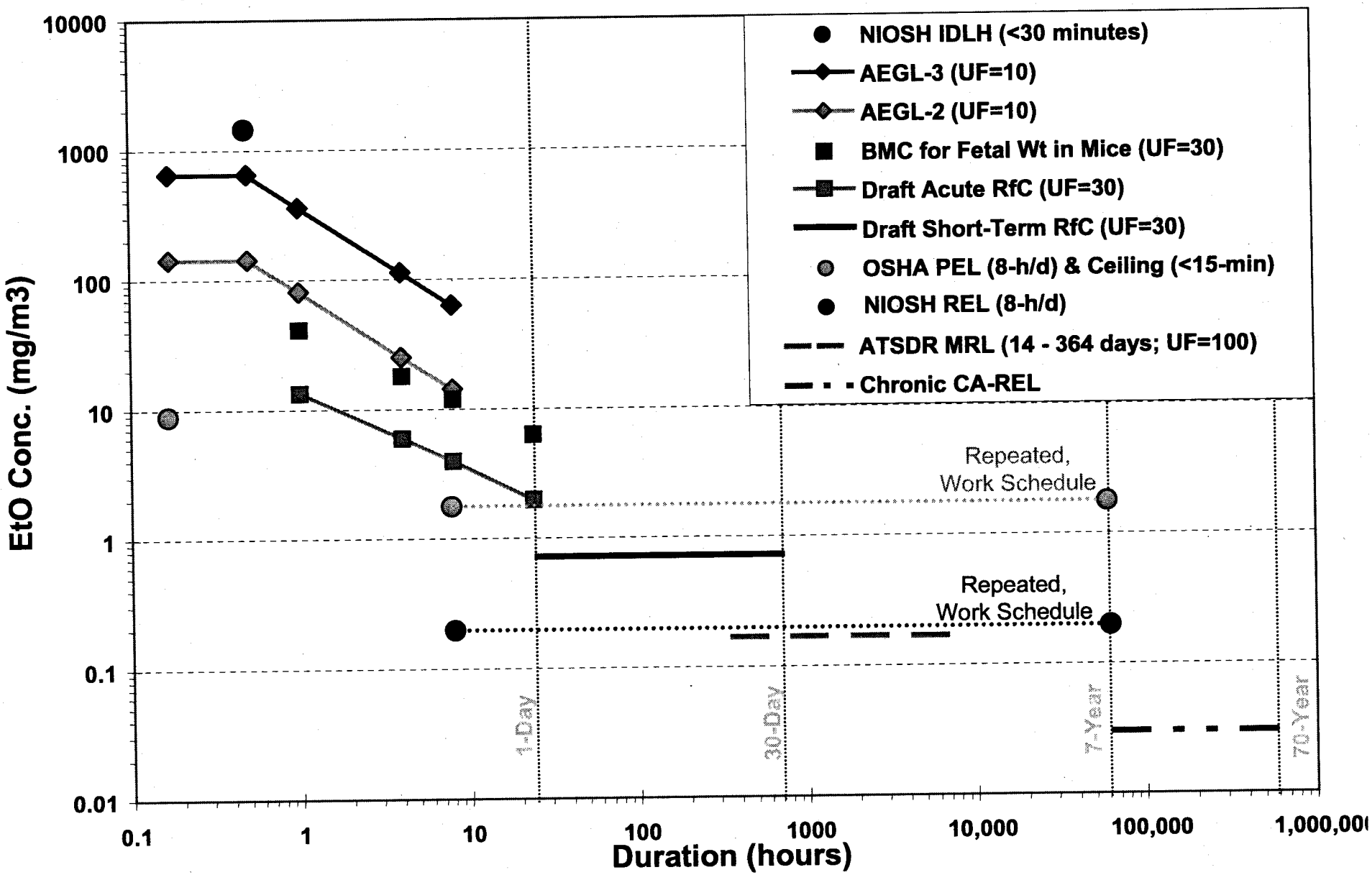
Background

- Most Developmental/Reproductive Toxicity studies are for repeated exposures
  - Often only data available for this endpoint (especially for inhalation)
  - How relevant to single exposures?
- Experience with Ethylene Oxide
  - Lead to interest in a better understanding of the relevance of typical studies to single exposure scenario

Ethylene Oxide: Comparison of Reference Values (Log-Log Scale)



The Study

- Literature review of chemicals with Dev/Repro Tox
  - Repeated exposures
    - Often for multiple groups of gestational days (i.e. whole organogenesis, critical "windows" therein)
  - Single exposures
    - Multiple groups exposed on different gestational days

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Methodology

- Utilized EPA's Benchmark Software (version 1.4.1) in order to model the dose-response relationships
  - Weibull model was used for modeling embryolethality as a dichotomous endpoint
  - Model parameters
    - Power restricted to ≥ 1
    - BMR level of .25 (extra risk)
    - Confidence level of 0.95

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Methodology (cont.)

- Hill model was used for modeling embryolethality as a continuous endpoint
- Model parameters
  - Power restricted to ≥ 1
  - BMR level of 2 control standard deviations (as estimated by model)
  - Confidence level of 0.95
  - Variance modeled as non-homogenous
- Nested models were not utilized due to lack of information on individual animals (all data modeled were summary information as presented in peer-reviewed articles)

Methodology (cont.)

- Measure of embryolethality:
  - As a dichotomous endpoint:
    - $\frac{\text{Number of resorptions and dead fetuses}}{\text{Number of implantations}}$
  - As a continuous endpoint:
    - Number of resorptions and dead fetuses per litter (values given as mean ± SD)

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Methodology (cont.)

- For all studies:
  - Rats were utilized
    - Wistar – Ema, et al. studies
    - Sprague-Dawley – Saillenfait et al. studies
  - Oral gavage was the route of exposure
  - Control animals were vehicle controls (olive or mineral oil)
  - Dams sacrificed on GD 20 or 21

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Methodology (cont.)

- Specific studies utilized for ButylBenzyl Phthalate analyses:
  - Ema, et al., 1992. Toxicol Letters 61, 1-7.
    - Gestational days 7-15
  - Ema, et al., 1993. Toxicology 79, 11-19.
    - Gestational days 7-9, 10-12, and 13-15
  - Ema, et al., 1999. J. Appl. Toxicology 19, 357-365.
    - Gestational days 13, 14, and 15

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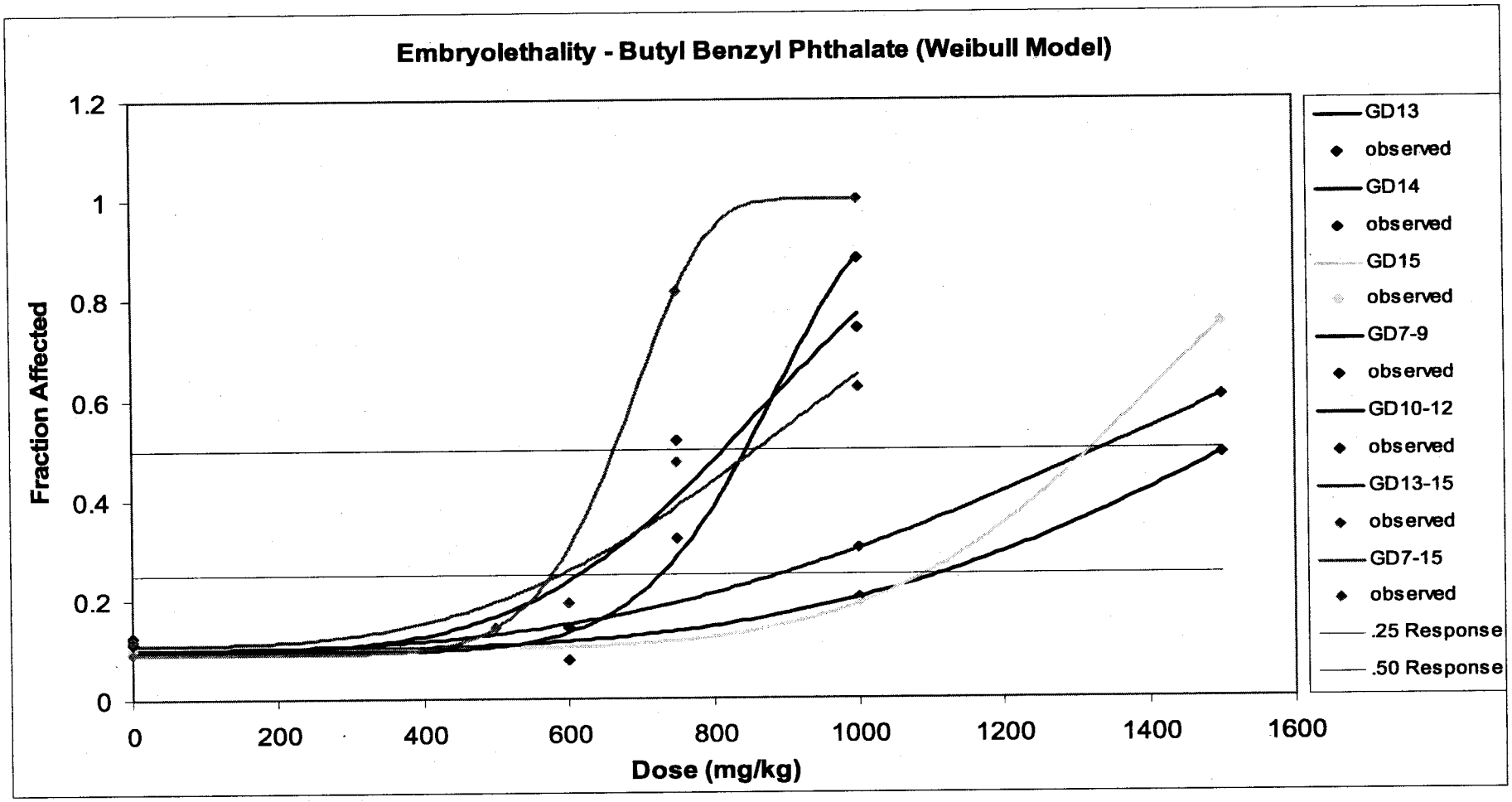
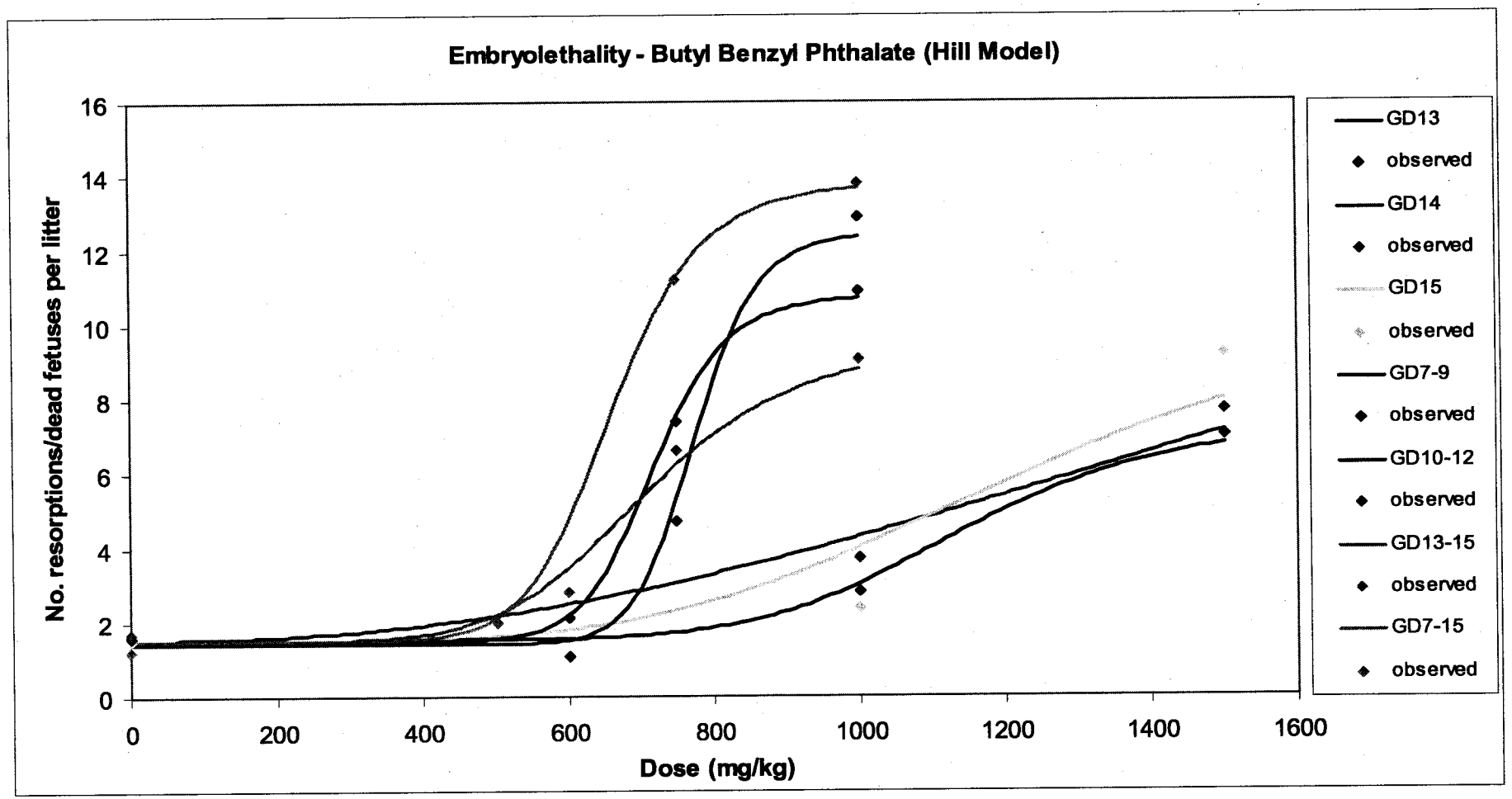
Methodology (cont.)

- Specific studies utilized for DiButyl Phthalate analyses:
  - Ema, et al., 1993. Toxicol Letters 69, 197-203.
    - Gestational days 7-15
  - Ema, et al., 1994. Toxicology 89, 163-174.
    - Gestational days 7-9, 10-12, 13-15
  - Saillenfait, et al., 1998. Toxicol Sci 45, 212-224.
    - Gestational day 14


Embryolethality - Butyl Benzyl Phthalate (Weibull Model)
Fraction Affected
1.2
1
0.8
0.6
0.4
0.2
0
0
200
400
600
800
1000
1200
1400
1600
Dose (mg/kg)
GD13
observed
GD14
observed
GD15
observed
GD7-9
observed
GD10-12
observed
GD13-15
observed
GD7-15
observed
.25 Response
.50 Response


Embryolethality - Butyl Benzyl Phthalate (Hill Model)
No. resorptions/dead fetuses per litter
0
2
4
6
8
10
12
14
16
0
200
400
600
800
1000
1200
1400
1600
Dose (mg/kg)
GD13
observed
GD14
observed
GD15
observed
GD7-9
observed
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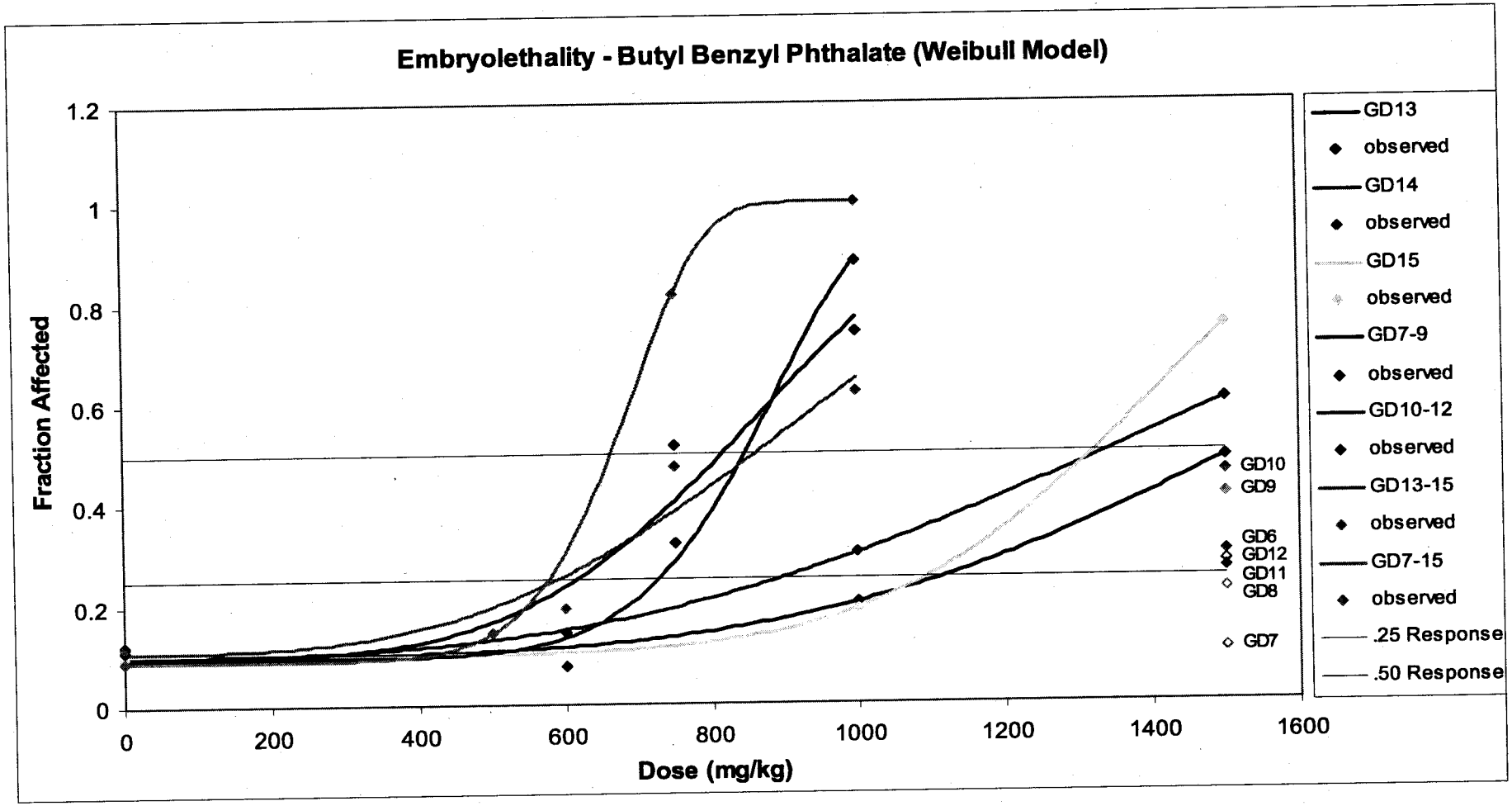
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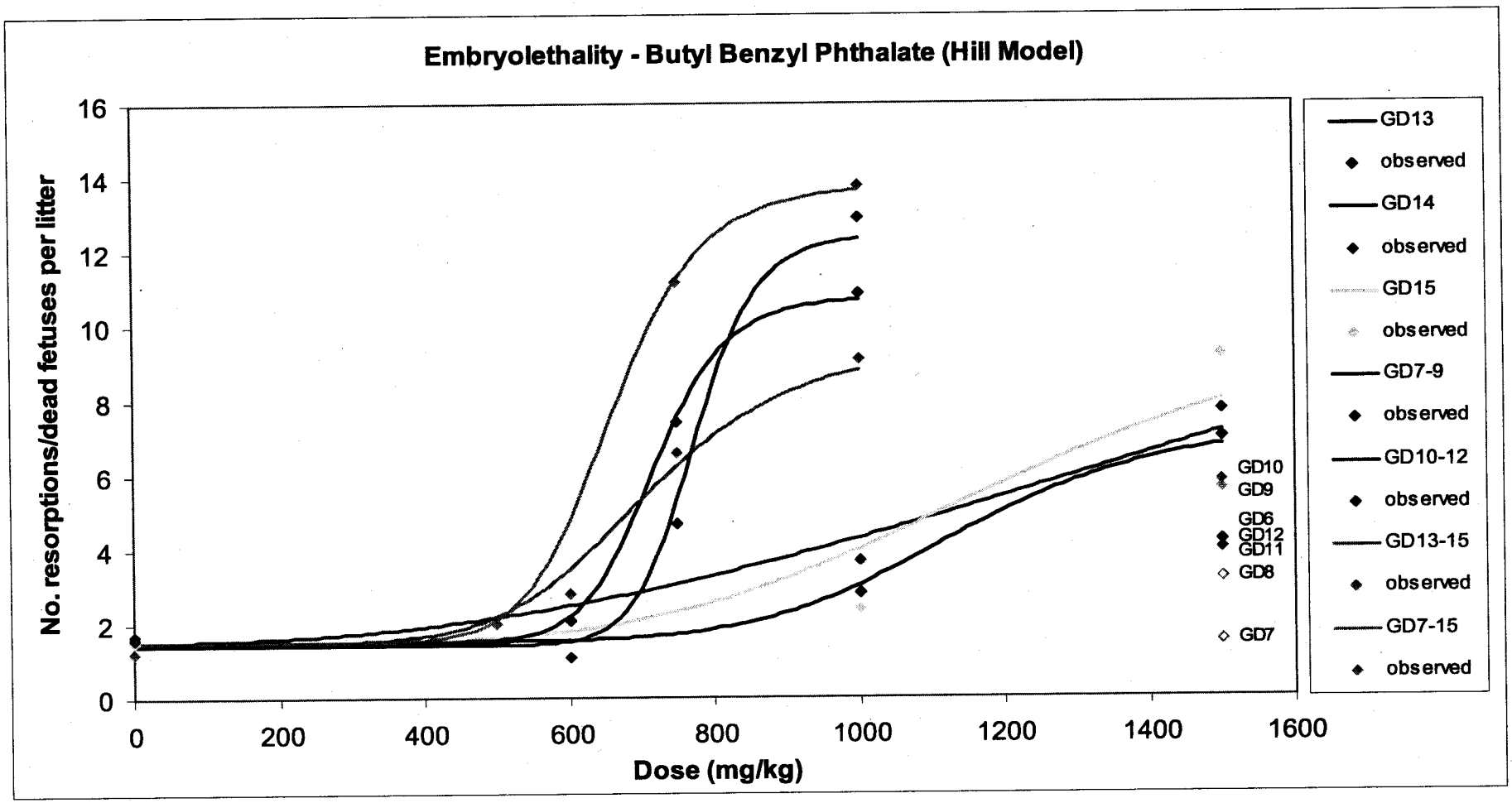
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GD13-15
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GD7-15
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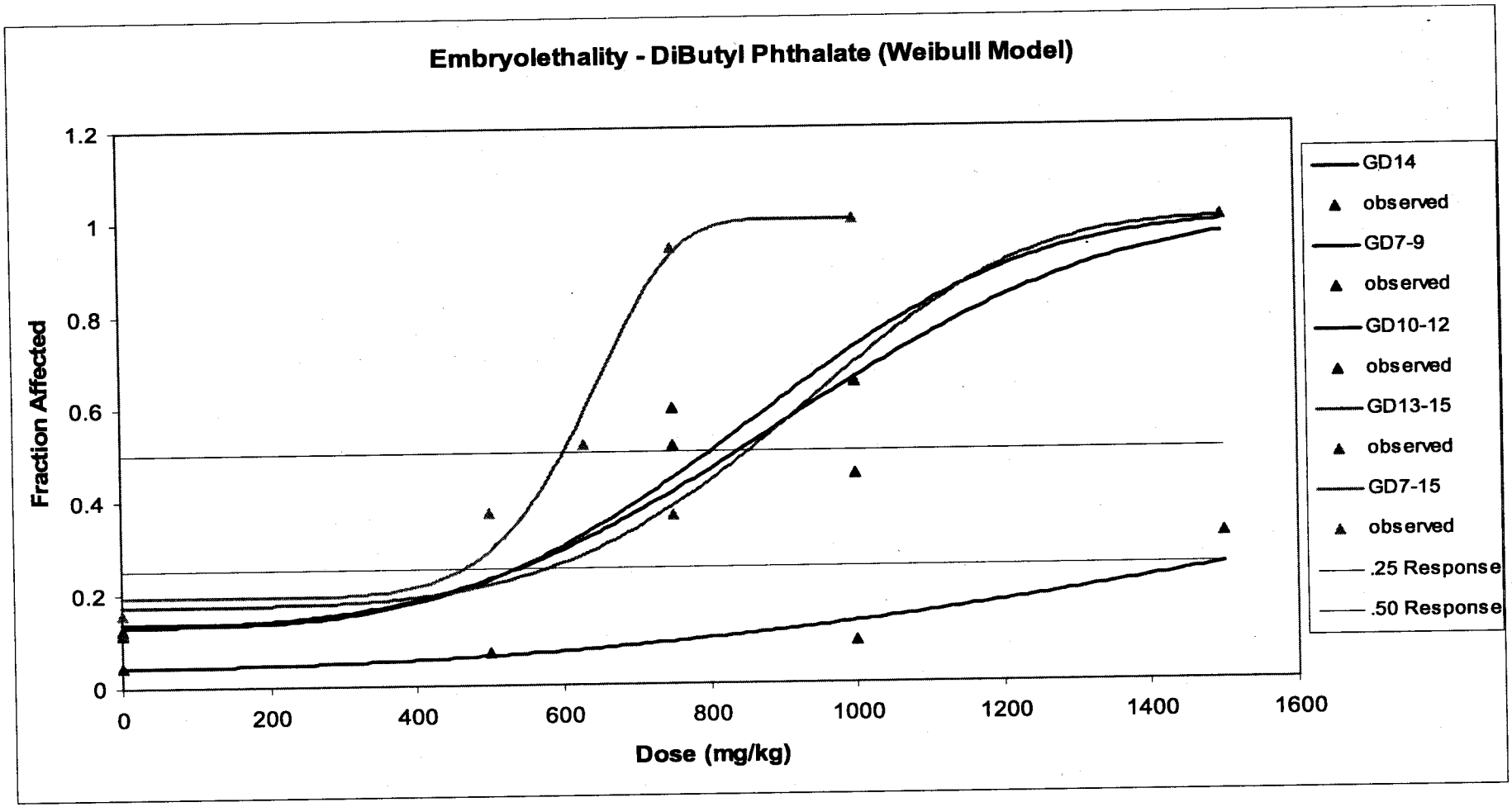
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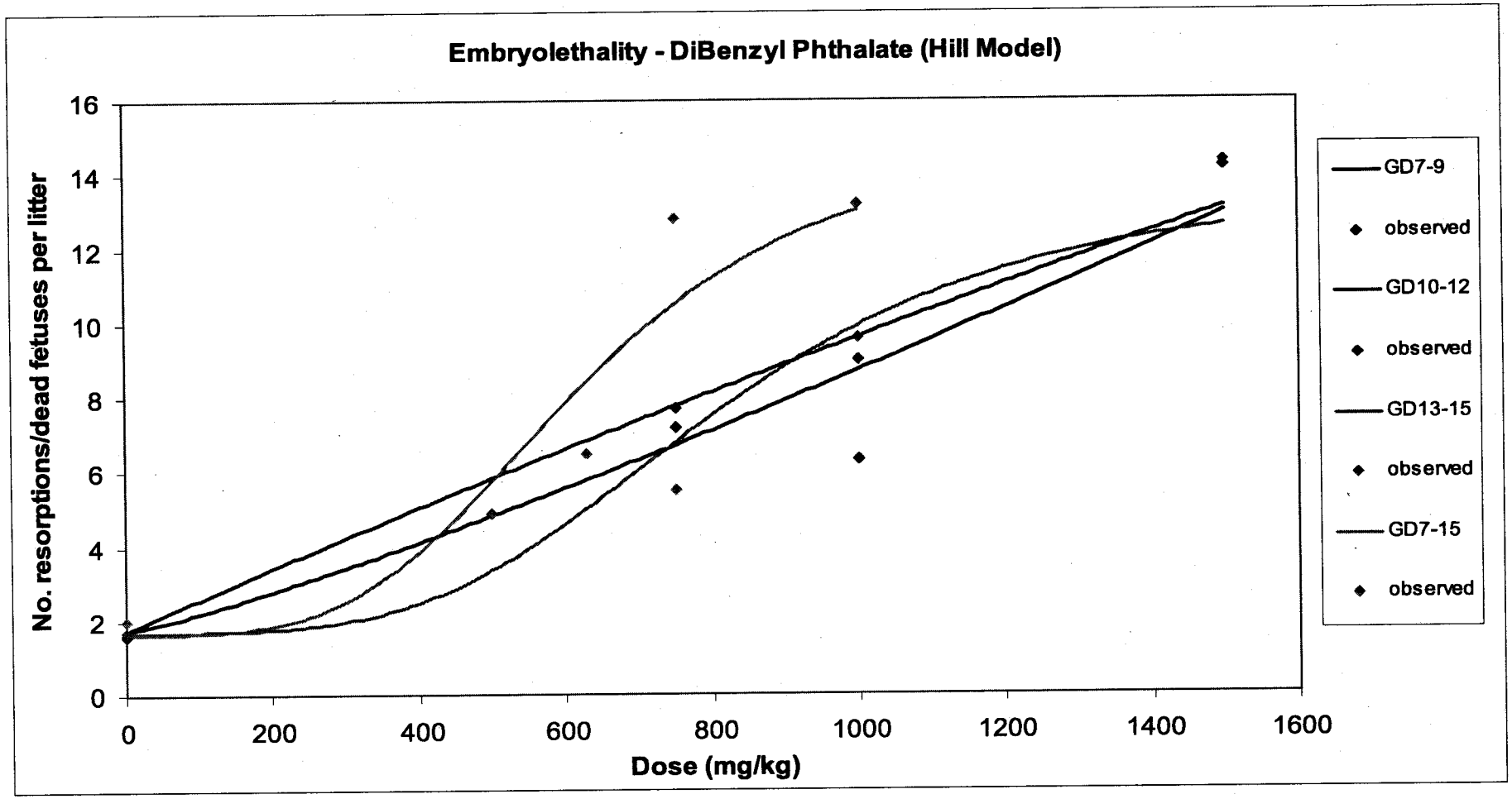
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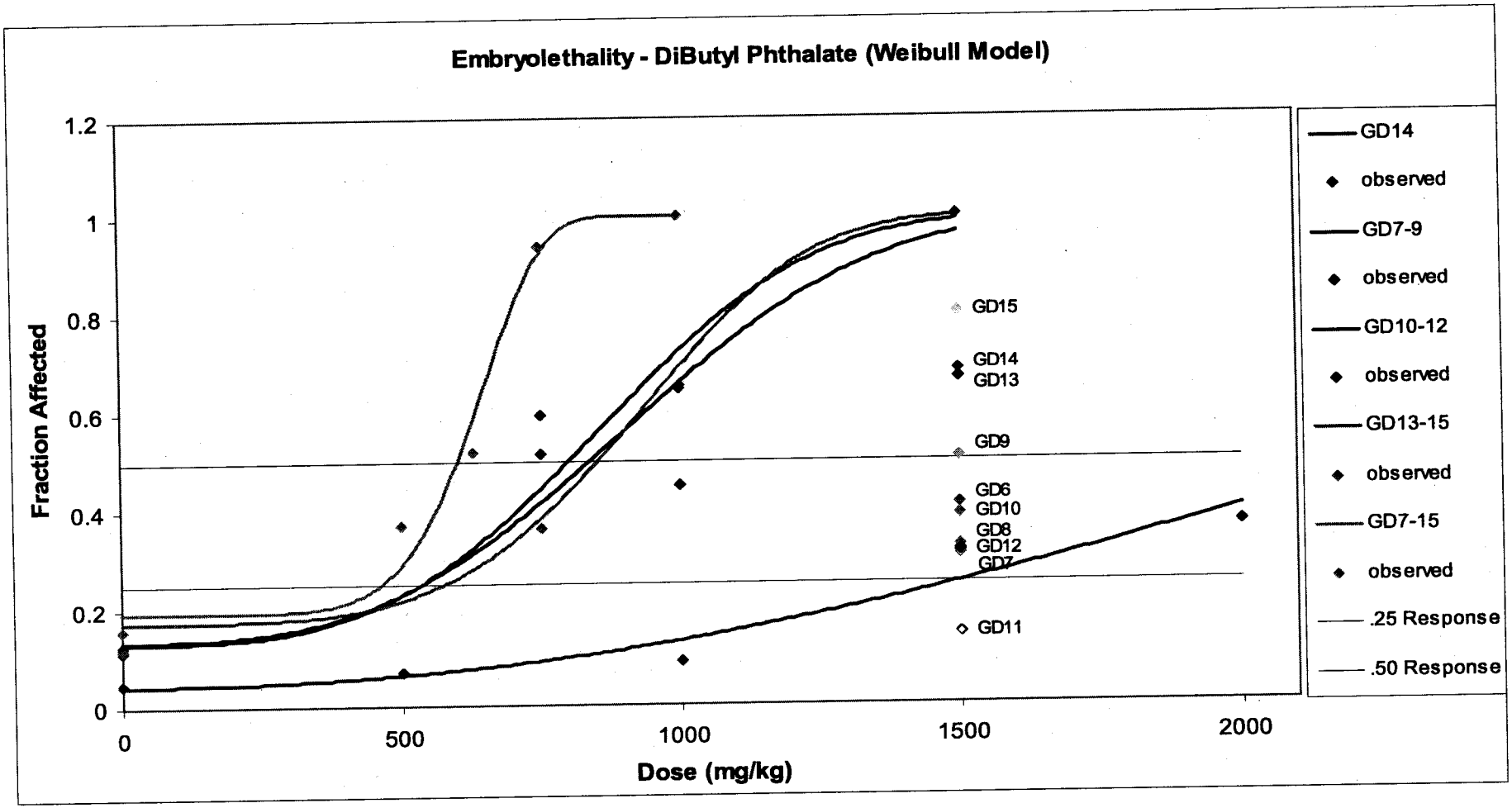
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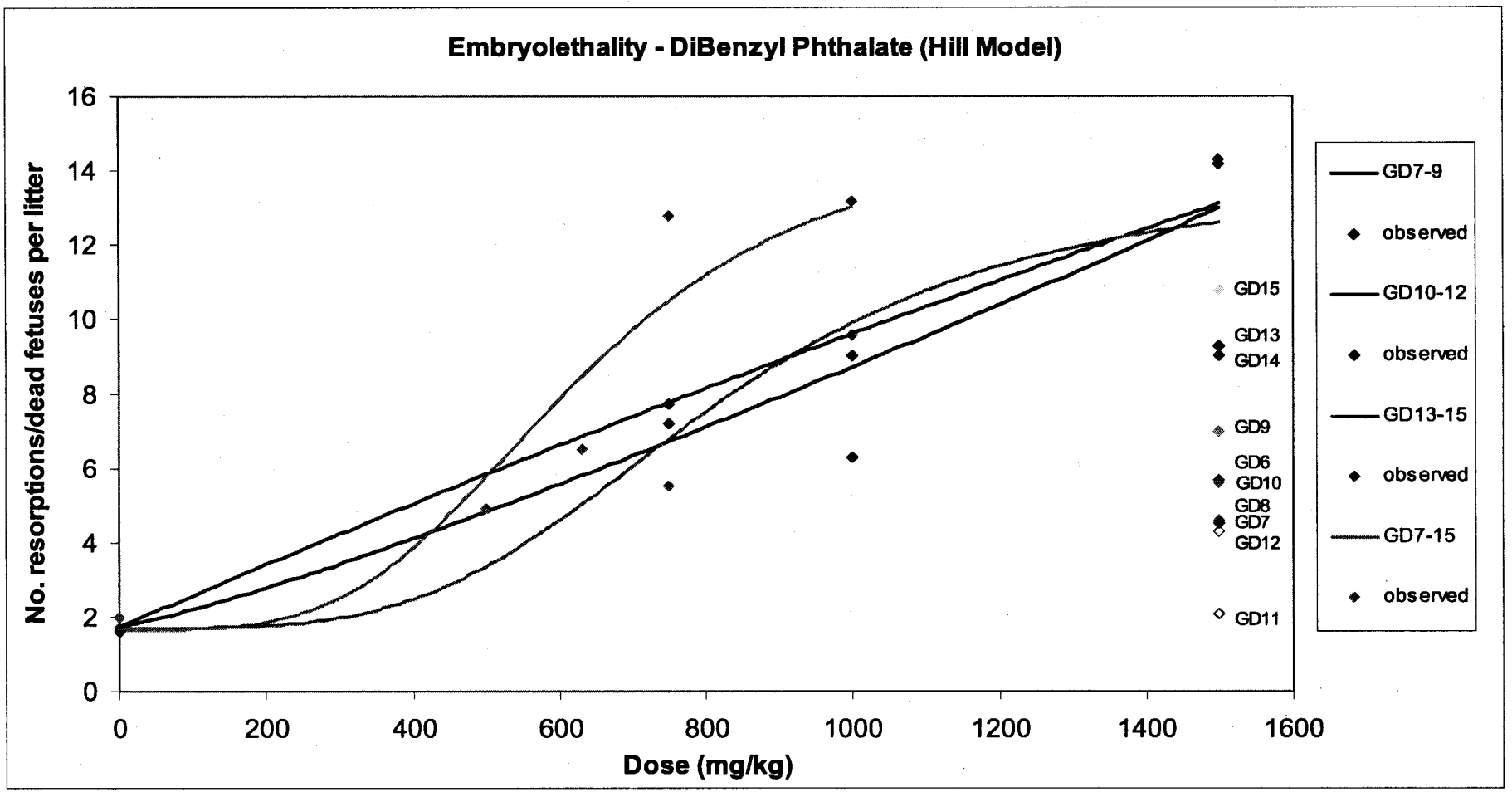
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Preliminary Conclusions & Points for Discussion

- Critical day of exposure may be a confounder in comparing single exposures to repeated exposures data
  - Which days of repeated exposure compared to which day for single exposure
- Number of repeated exposures is often a factor
  - Cumulative effects
  - Clearance of compound and/or recovery from the effect
- Comparisons of dose-response curves may provide more information than point estimates (e.g., NOAEL +/or LOAEL)
- Are the oral gavage studies relevant to inhalation exposures?