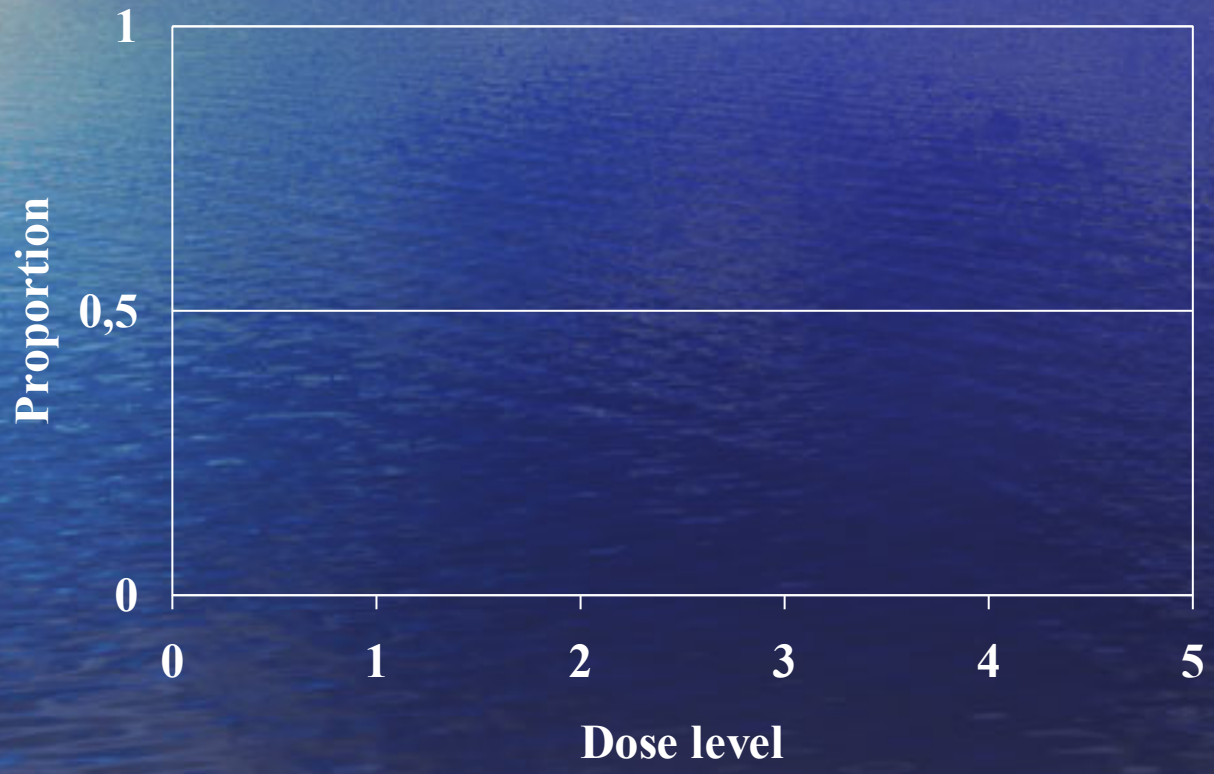
Development of Response Surface Pathway Design (RSP) in Laboratory Animals

Professor Stig Larsen
Norwegian University of Life Sciences
Faculty of Veterinary Medicine and Biosciences

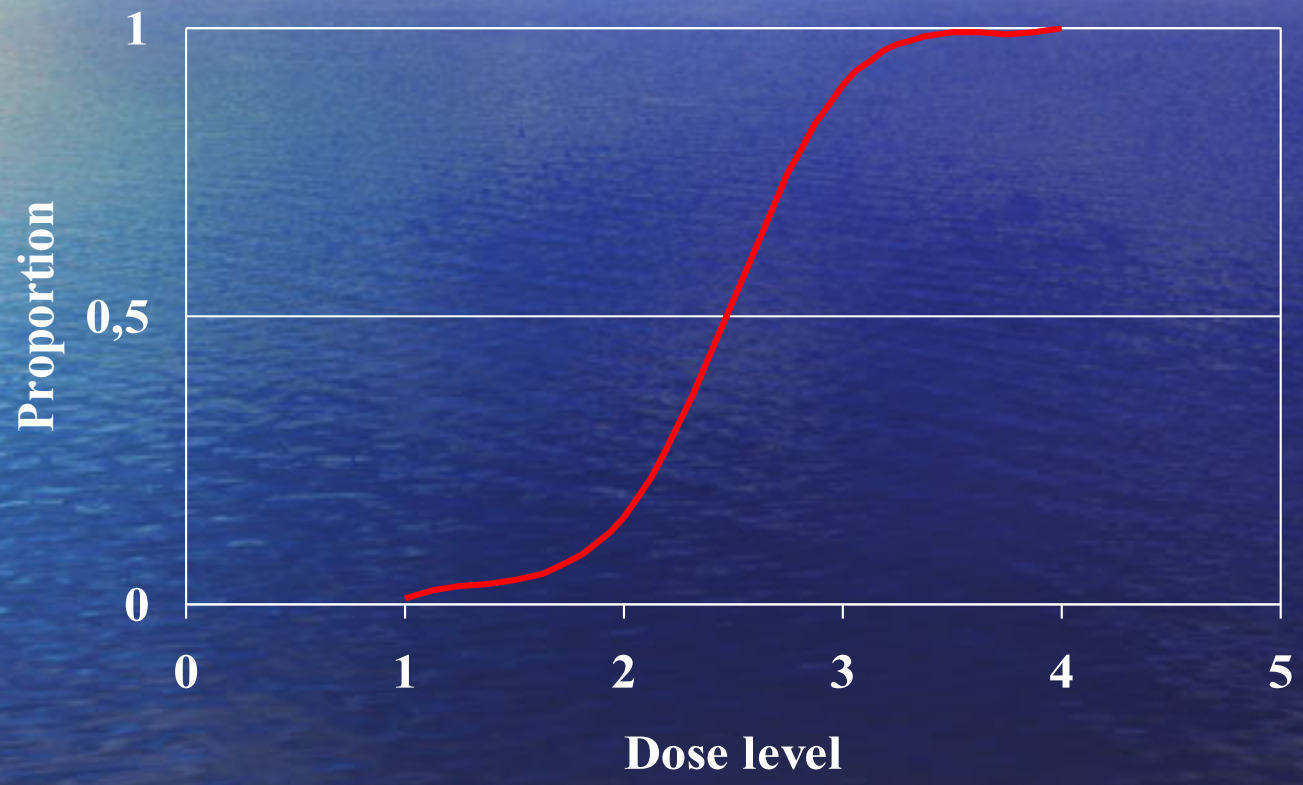
The stochastic nature in biology

- This is not commonly taken into account in the planning phase of clinical trials.
- Everything has to be strictly planned and stated in the trial protocol before start of the study.
- Whatever to be observed during the study, the protocol procedures is mainly to be kept unchanged.

Selection of LD50-doses



Selection of LD50-doses



Dose design in Laboratory Animal studies

- **Classic LD_{50} design:** Trevan 1927; still in used today.
- **Up-and- Down design :** Dixon and Mood 1948; most commonly in use from 1960th.
- **Up-and-Down Procedure (UPD)**: Bruce 1985; standard method and recommended by OECD from 2002
- **Random Walk design (RW)**: Tsutakawa 1967; Block UPD method and included in OECD
- **3+3 or A+B- design:** Storer 1989 and Lin 2001; commonly in use from 2007
- **Basic Response Surface Pathway design (RSP):** Aune and Larsen 2007

Type of Designs in Dose-finding studies

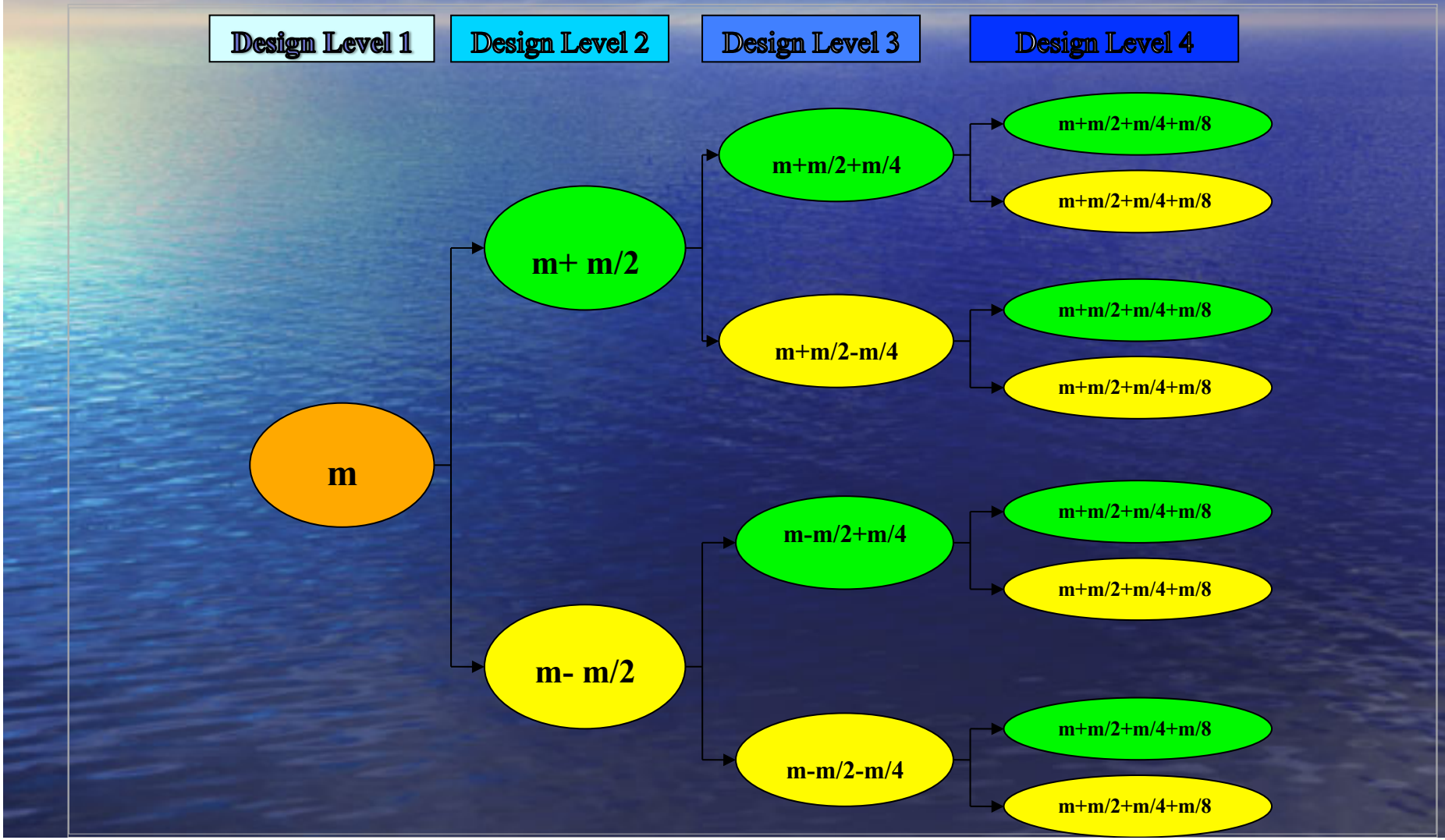
Rule based design

- Classic LD50 , UPD, RW, 3+3, RSP

Statistical based design

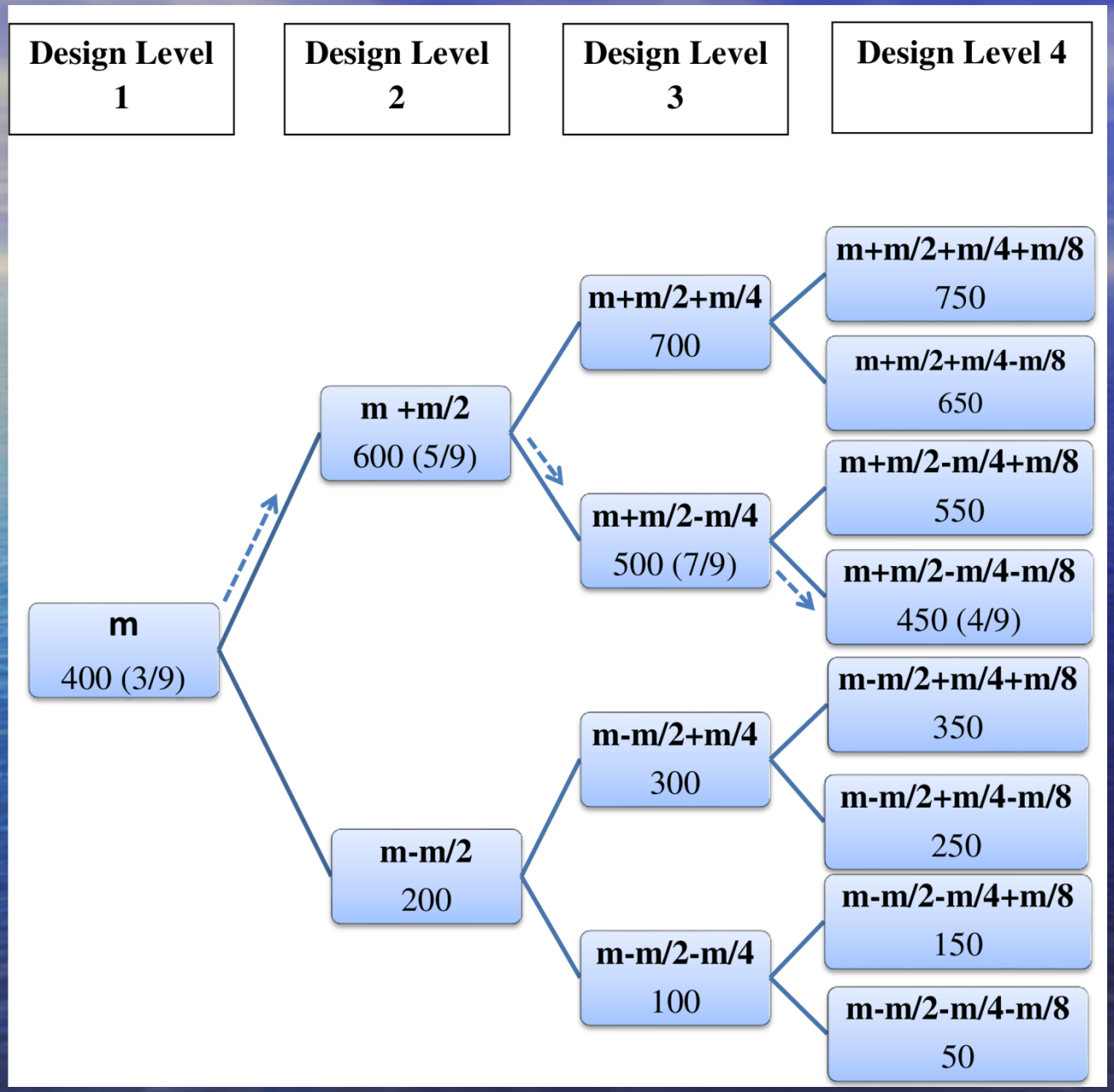
- Bayesian

A general four level Response Surface Pathway Design



Materials & Methods

- Priory knowledge: The LD_{50} Yessotoxin window is 100-700 μg/kg. bw.
- Mouse strain: Male ICR
- Weight: 19-22.5g
- Number of dose levels: 4
- Starting dose in the response surface design: 400 μg/kg. bw.


Design Level 1
Design Level 2
Design Level 3
Design Level 4
m
400 (3/9)
m +m/2
600 (5/9)
m-m/2
200
m+m/2+m/4
700
m+m/2-m/4
500 (7/9)
m-m/2+m/4
300
m-m/2-m/4
100
m+m/2+m/4+m/8
750
m+m/2+m/4-m/8
650
m+m/2-m/4+m/8
550
m+m/2-m/4-m/8
450 (4/9)
m-m/2+m/4+m/8
350
m-m/2+m/4-m/8
250
m-m/2-m/4+m/8
150
m-m/2-m/4-m/8
50

K-Adjustment factor

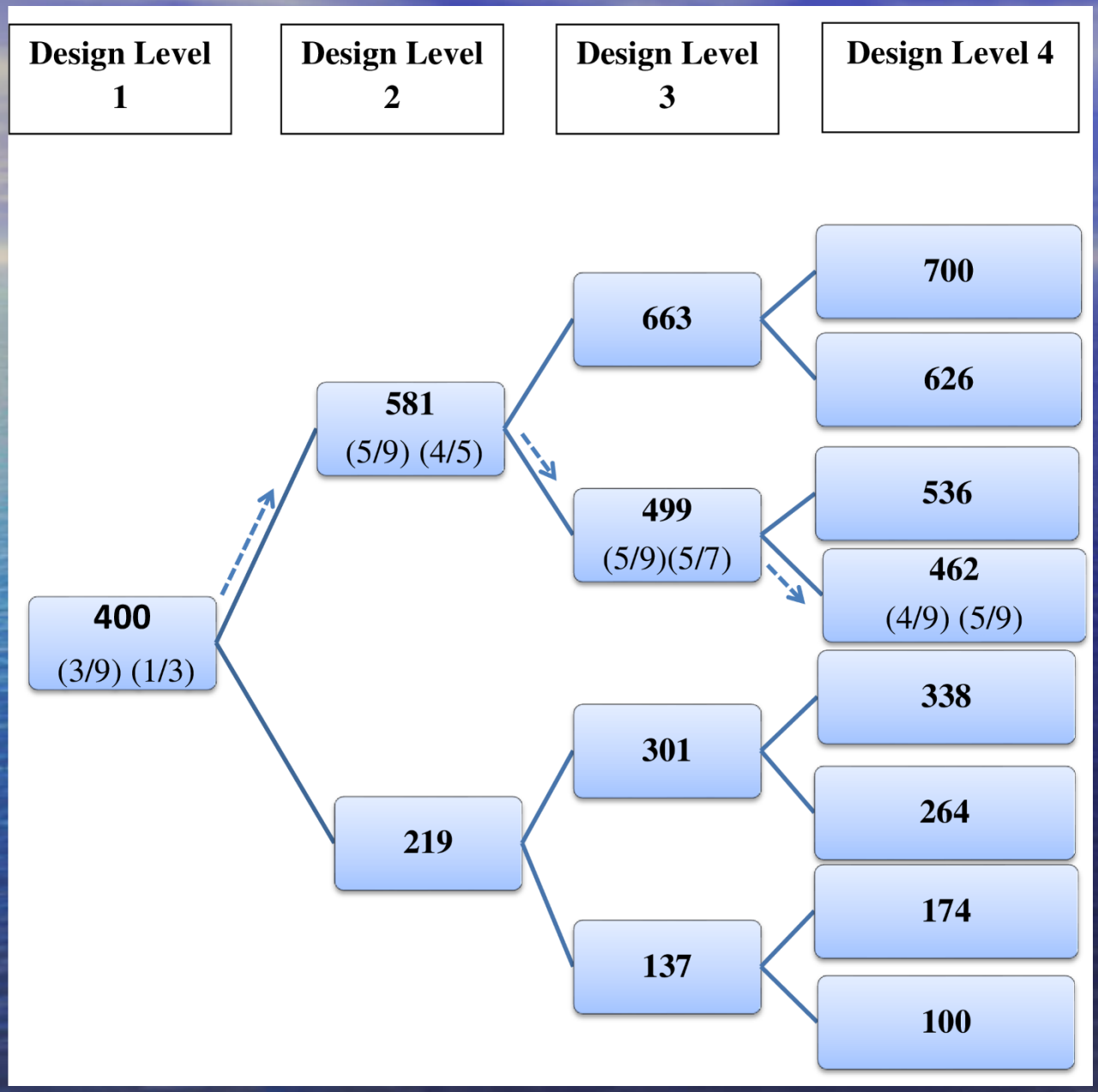
$$m_i = m_{i-1} \pm \frac{m}{2^{i-1}}$$

$$m_i = m_{i-1} \pm \frac{m}{k^{i-1}}$$

Calculation of K-Factor

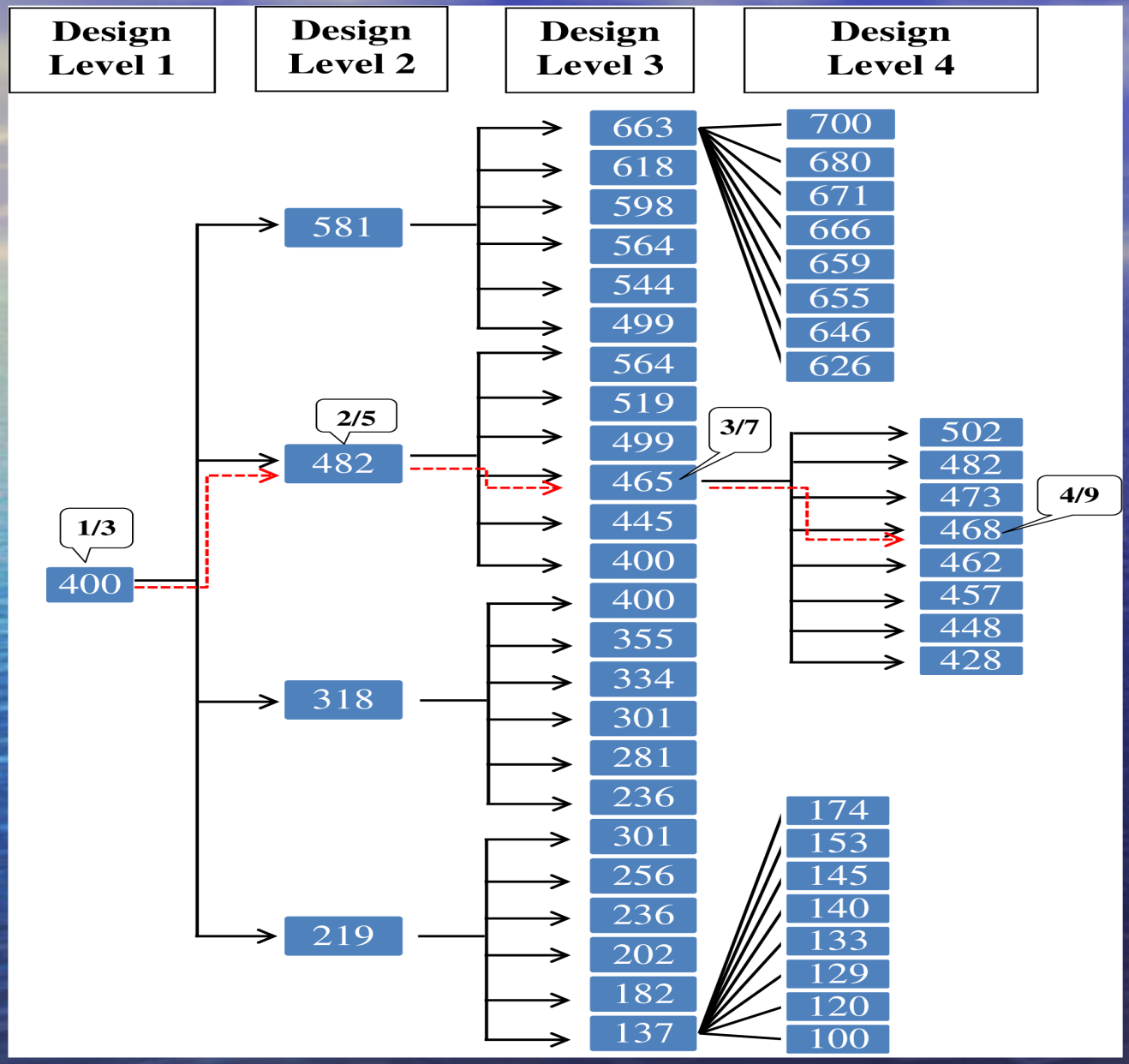
Upper maximum dose = $D_U = m + m/k + m/k^2 + m/k^4 + m/k^8 = 700$

Lower minimum dose = $D_L = m - m/k - m/k^2 - m/k^4 - m/k^8 = 100$


Design Level 1
Design Level 2
Design Level 3
Design Level 4
400
(3/9) (1/3)
581
(5/9) (4/5)
219
663
499
(5/9)(5/7)
301
137
700
626
536
462
(4/9) (5/9)
338
264
174
100

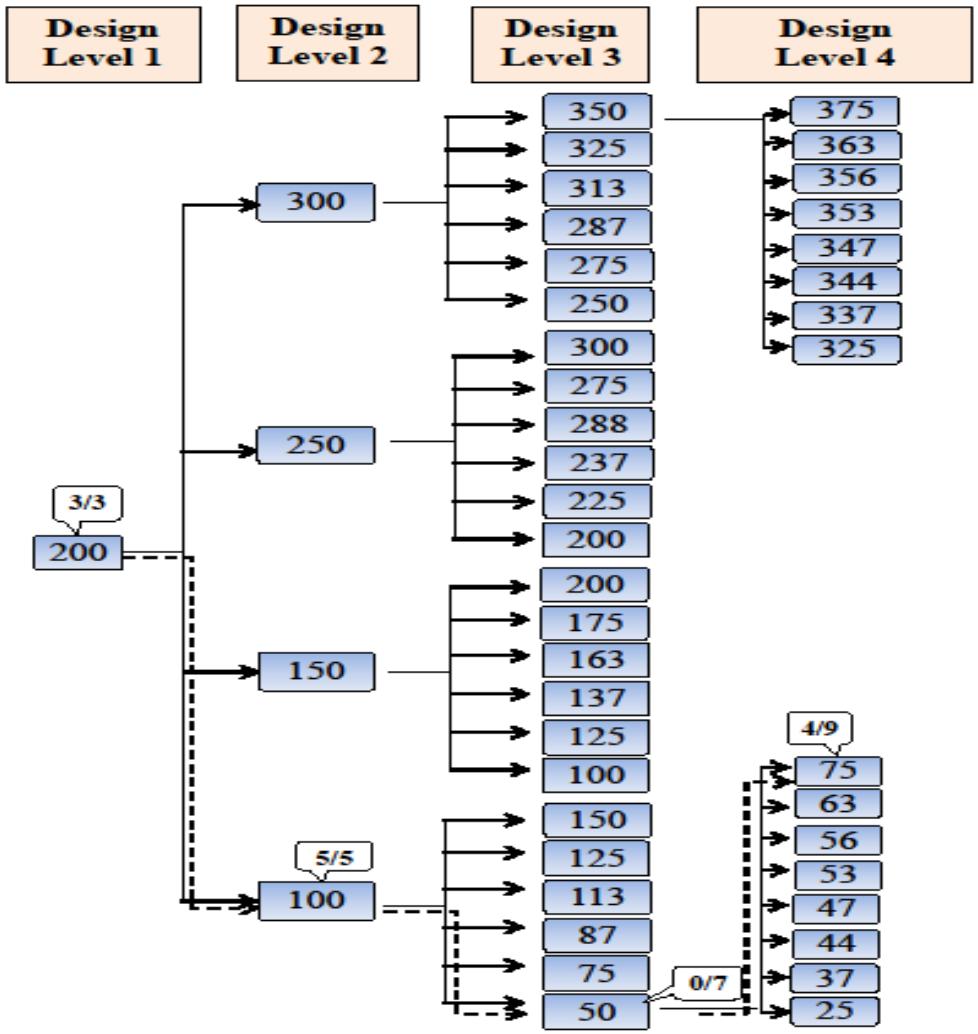
From Binomial to multinomial outcome to determine dose levels

Number dead	Level 2 (m_2)	Level 3 (m_3)	Level 4 (m_4)	Level 5 (m_5)
0	$m_1 + m_1/k$	$m_2 + m_1/k^2$	$m_3 + m_1/k^3$	$m_4 + m_1/k^4$
1	$m_1 + m_1/k^2$	$m_2 + m_1/k^3$	$m_3 + m_1/k^4$	$m_4 + m_1/k^5$
2	$m_1 - m_1/k^2$	$m_2 + m_1/k^4$	$m_3 + m_1/k^5$	$m_4 + m_1/k^6$
3	$m_1 - m_1/k$	$m_2 - m_1/k^4$	$m_3 + m_1/k^6$	$m_4 + m_1/k^7$
4		$m_2 - m_1/k^3$	$m_3 - m_1/k^6$	$m_4 + m_1/k^8$
5		$m_2 - m_1/k^2$	$m_3 - m_1/k^5$	$m_4 - m_1/k^8$
6			$m_3 - m_1/k^4$	$m_4 - m_1/k^7$
7			$m_3 - m_1/k^3$	$m_4 - m_1/k^6$
8				$m_4 - m_1/k^5$
9				$m_4 - m_1/k^4$


Design Level 1
Design Level 2
Design Level 3
Design Level 4
1/3
400
581
2/5
482
318
219
663
618
598
564
544
499
564
519
499
465
445
400
400
355
334
301
281
236
301
256
236
202
182
137
700
680
671
666
659
655
646
626
3/7
502
482
473
468
462
457
448
428
4/9
174
153
145
140
133
129
120
100

Estimation of LD_{50} of AZA1

- The dose window for AZA1in mouse is 25-375 µg/kg. bw.
- Mouse strain to be used : Female NMRI mice
- Mouse weight: 15 – 21 g
- Number of design-levels : 4
- Number of mice on design is 3, 5, 7 and 9
- Start dose in the RSP-design is chosen to (375+25)/2 = 200 µg/kg. bw.
- The dose adjustment k-factor calculated to k=2


Design Level 1
Design Level 2
Design Level 3
Design Level 4
3/3
200
300
250
150
5/5
100
350
325
313
287
275
250
300
275
288
237
225
200
200
175
163
137
125
100
150
125
113
87
75
50
375
363
356
353
347
344
337
325
4/9
75
63
56
53
47
44
37
25
0/7

Accuracy and needed sample size in estimation of LD_{50} using basic- and developed RSP.

Design	Dose (µg/kg BW)	Proportion of dead mice	LD_{50} with 95% CI (µg/kg BW)	No of mice needed
Basic RSP	400 450 500 600	3/9 4/9 7/9 5/9	463 (383 – 535) [152]	**N=36**
***) Optimising use of mice**	400 462 499 581	1/3 5/9 5/7 4/5	447 (378 – 504) [126]	**N=24** **{n=16}**
***) Multinomial decision variable with all four levels**	**400** **465** **468** **482**	**1/3** **3/7** **4/9** **3/5**	**473** **(442 – 517)** **[75]**	**N=15** **{n=9}**