Analysis of the Embryo-fetal Developmental Toxicity Studies with Glyphosate

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- Analysis of Results in the Rabbit
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- Classification

Studies

- In this presentation:
  - Only consideration is given to the results of embryo-fetal development toxicity studies that have been performed with technical glyphosate (glyphosate acid)
  - Results of embryo-fetal development toxicity studies are considered that are in compliance with internationally agreed test guidelines (OECD TG 414) applicable at the time of conduct of the study and that are performed in accordance with good laboratory practices (GLP)
  - Some less compliant studies that are considered by the RMS as “supplementary” are also used for the toxicological evaluation but are given less weight in the weight-of-evidence analysis

Analysis of Results in the Rat

Embryo-fetal Developmental Toxicity Studies of Glyphosate in the Rat

Study	*Strain*	*No of gravid animals per group*	*Duration of treatment (GD)*	*Doses (mg/kg bw/day)*	*Comment*
Moxon (2002)	Alpk:APfSD Wistar-derived	22-24	6-15	0-250-500-1000	
Wood (1996)	Sprague-Dawley	22-25	6-15	0-100-500-1000	Not in RAR
Hatakenaka (1995)	Sprague-Dawley Crj:CD	22-24	6-15	0-30-300-1000	
Brooker *et al*. (1991)	Sprague-Dawley	23-25	6-15	0-300-1000-3500	Not in RAR
Suresh (1991)	Wistar	20-30	6-15	0-1000	Not in compliance with OECD TG (only one dose group), not in RAR
Tasker *et al*. (1980)	Sprague-Dawley COBS CD	20-23	6-19	0-300-1000-3500	Pre-GLP and pre-OECD TG study, not in RAR

Analysis of the Results in the Rat:
Maternal Toxicity

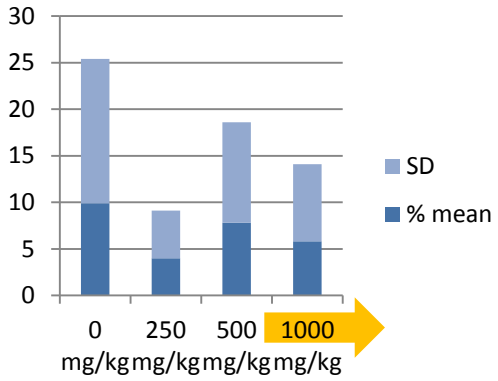
Moxon, 2002 Mortality/Clinical signs	Dose (mg/kg)			
	0	**250**	**500**	**1000**
Mortality[a]	1/22[b]	0/24	0/23	0/24
Food consumption (GD19-26, % change)	-	↗7.1*	↗3.4	↗3.4
Body weight (GD22, % change)	-	↗0.9	↗1.2	↗0.5
Clinical signs	None	None	None	None

Hatakenaka, 1995 Mortality/Clinical signs	Dose (mg/kg)			
	0	**30**	**300**	**1000**
Mortality[a]	0/23	0/24	0/24	0/22
Food consumption (GD)	-	No change	No change	↘(6-9) ↗(15-20)
Body weight	-	No change	No change	No change
Loose stools	0/22	0/22	0/22	20/22

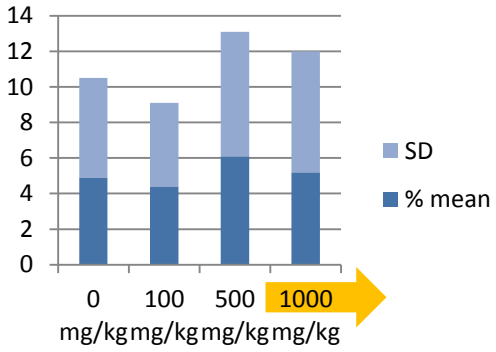
*: $p<0.05$; a: Spontaneous deaths and pre-term sacrifice; b: maldosing

Analysis of the Results in the Rat:
Mean % Post-implantation Loss

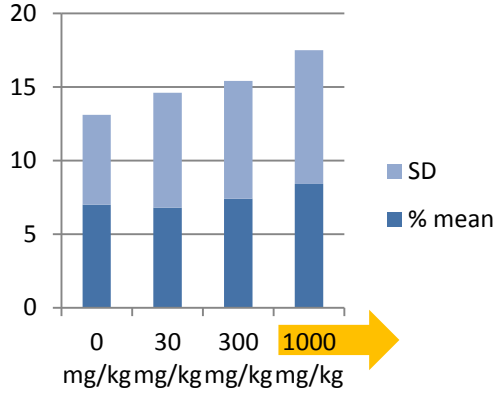
Moxon, 2002



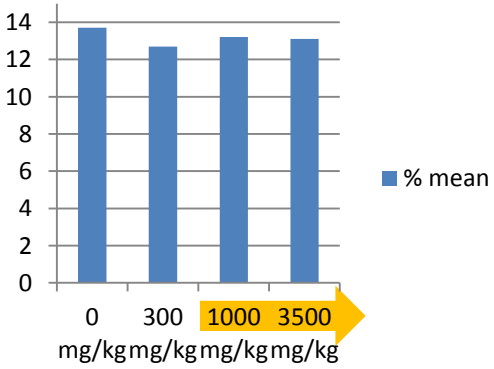
Wood, 1996



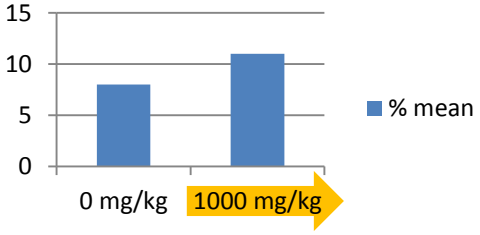
Hatakenaka, 1995



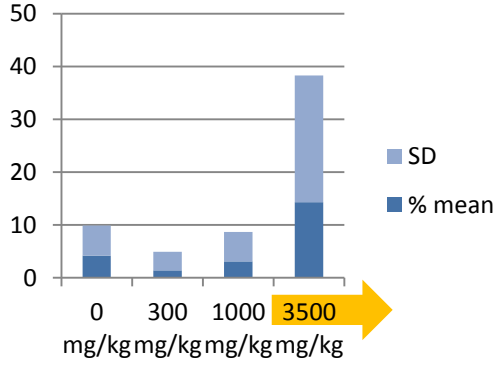
Brooker *et al*, 1991



Suresh, 1991

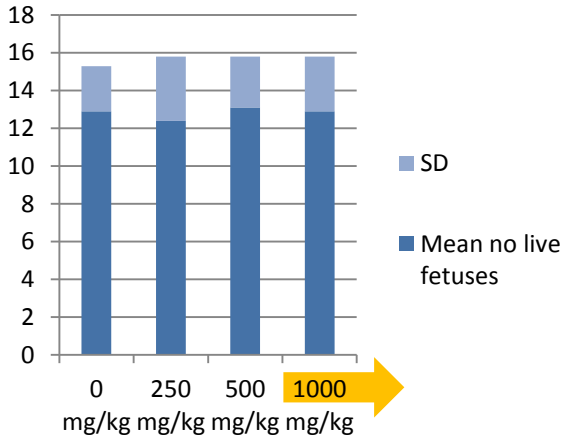


Tasker *et al*., 1980

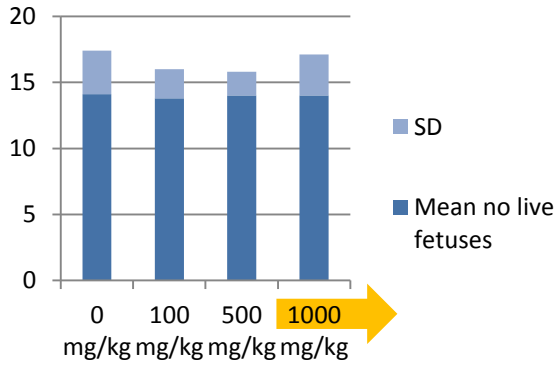


Analysis of the Results in the Rat:
Mean Number of Live Fetuses/Litter

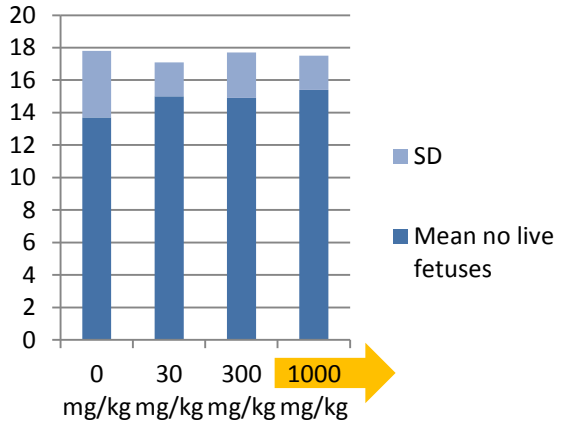
Moxon, 2002



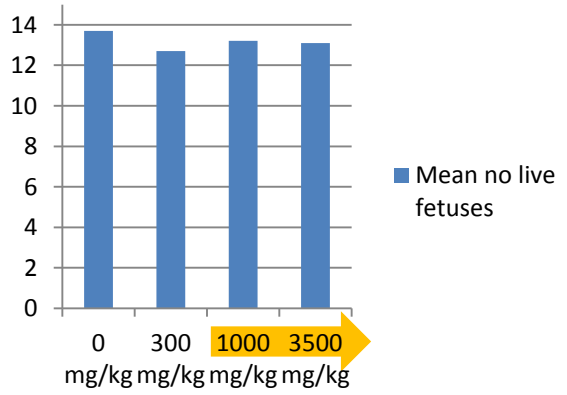
Wood, 1996



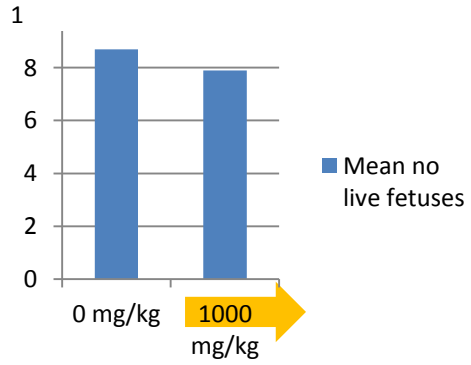
Hatakenaka, 1995



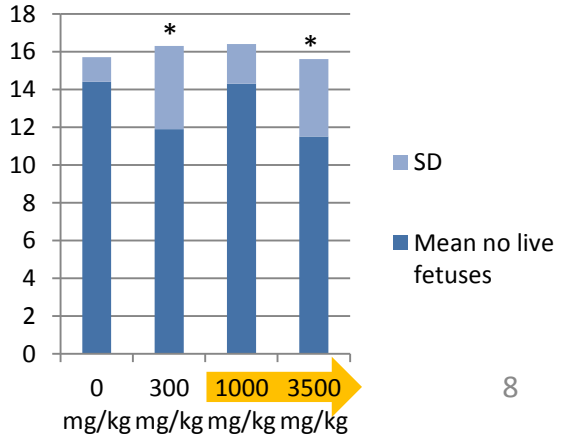
Brooker *et al*, 1991



Suresh, 1991

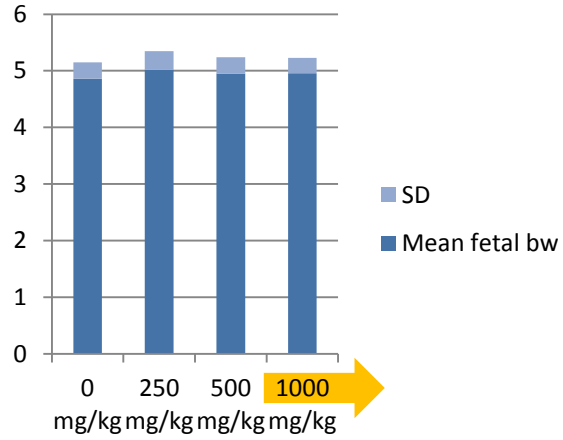


Tasker *et al*., 1980

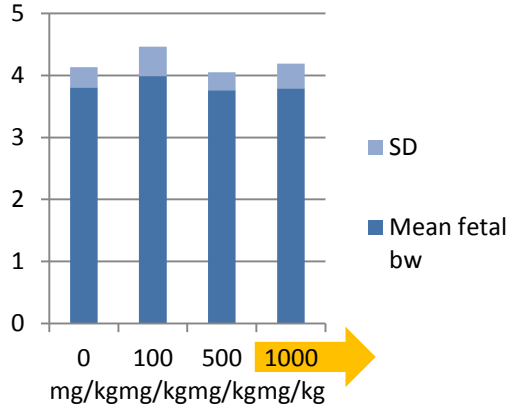


Analysis of the Results in the Rat:
Mean Fetal Body Weight (g)

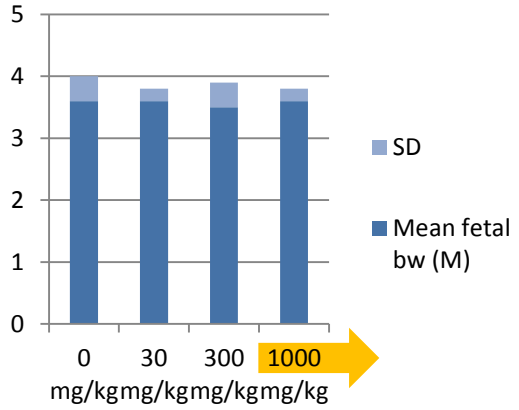
Moxon, 2002



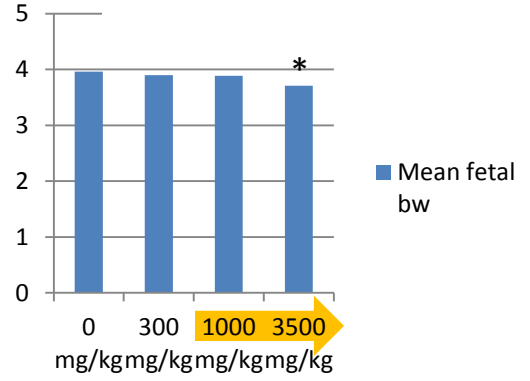
Wood, 1996



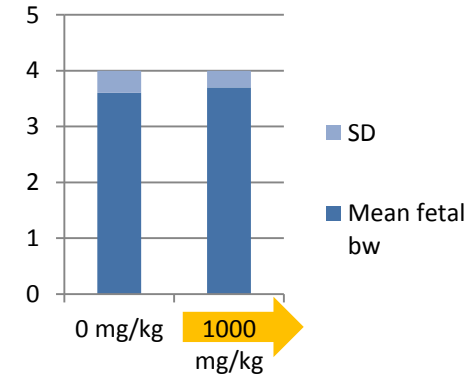
Hatakenaka, 1995



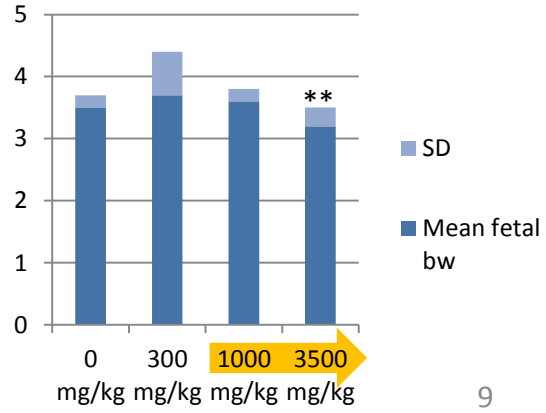
Brooker *et al*, 1991



Suresh, 1991



Tasker *et al*., 1980



Analysis of the Results in the Rat:
Number of malformed Fetuses (Number of Litters with malformed Fetuses)

Study	*0 mg/kg*	*30 mg/kg*	*100 mg/kg*	*250 mg/kg*	*300 mg/kg*	*500 mg/kg*	*1000 mg/kg*	*3500 mg/kg*
Moxon (2002)	1(1)			1(1)		1(1)	2(2)	
Wood (1996)	3(3)		1(1)			0(0)	0(0)	
Hatakenaka (1995)	2(1)	1(1)			3(2)		5(2)	
Brooker *et al*. (1991)	1(1)				2(2)		1(1)	3(2)
Suresh (1991)	5(5)[a] 17(8)[b]						0[a] 10(6)[b]	
Tasker *et al*. (1980)	3(3)				0(0)		0(0)	10(3)[c]

a: external /visceral malformations; b: skeletal malformations; c: includes 6 fetuses in one litter with a syndrome of bent tail, open eyelids, missing kidneys and ureters, and various skeletal defects and 3 fetuses in another litter with dwarfism. All malformations were seen in historical controls.

Analysis of the Results in the Rat:
Number of fetuses with Cardio-vascular Malformations

Study	*0 mg/kg*	*30 mg/kg*	*100 mg/kg*	*250 mg/kg*	*300 mg/kg*	*500 mg/kg*	*1000 mg/kg*	*3500 mg/kg*
Moxon (2002)	0			0		0	0	
Wood (1996)	1[a] 1[b]		1[a]			0	0	
Hatakenaka (1995)	0	0			1[a] 1[c]		1[a]	
Brooker *et al*. (1991)	0				0		1[a]	1[a]
Suresh (1991)	0						0	
Tasker *et al*. (1980)	0				0		0	0

a: Interventricular septal defect; b: Retro esophageal right-sided aortic arch; c: Right aortic arch

Embryo-fetal Developmental Toxicity Studies of Glyphosate in the Rat: Conclusions

- The NOAEL for maternal toxicity in the rat is 300 mg/kg bw/day, based on loose stools at 1,000 mg/kg bw/day (Hatakenaka, 1995)
- The NOAEL for embryo-fetal developmental toxicity in the rat is 1,000 mg/kg bw/day, based on a reduced mean fetal body weight at 3500 mg/kg bw/day (Brooker *et al*., 1991; Tasker et al., 1980)
- Overall, the rat developmental toxicity studies do not show any evidence of cardiovascular or other types of malformations as a result of glyphosate acid exposure via the oral route at doses of up to 3,500 mg/kg bw/day

Analysis of Results in the Rabbit

Embryo-fetal Developmental Toxicity Studies of Glyphosate in the Rabbit*

Study	*Strain*	*No of gravid animals per group*	*Duration of treatment (GD)*	*Doses (mg/kg bw/day)*	*Comment*
Coles and Doleman (1996)	New Zealand White	18	7-19	0-50-200-400	
Moxon (1995)	New Zealand White	20	7-19	0-100-175-300	
Hojo (1995)	Japanese white rabbits, Kbl:JW	18	7-19	0-10-100-300	
Suresh (1993)	New Zealand White	15-26	6-18	0-20-100-500	
Brooker *et al*. (1991)	New Zealand White	16-20	7-19	0-50-150-450	
Bhide and Patil (1989)	New Zealand White	15	6-18	0-125-250-500	Serious reporting deficiencies, not GLP
Tasker *et al* (1980)	Dutch Belted	16-17	6-27	0-75-175-350	Pre-GLP and pre-OECD TG study, only 6 litters at high dose

*: The Study of Bhide and Patil ,1989 has not been included in this assessment because of serious reporting deficiencies (no individual data, no statistical analysis, no uterine weights, no maternal necropsy results)

Analysis of the Results in the Rabbit:

Pre-term Maternal Mortality (%)*

Study	Dose (mg/kg)													
	0	10	20	50	75	100	150	175	200	300	350	400	450	500
Coles and Doleman (1996)	6[a]			0					6[a]			12		
Moxon (1995)	5					10		10		10				
Hojo (1995)	0	0				0				18				
Suresh (1993)	8[a]		0			25[b]								53
Brooker *et al.* (1991)	0			0			0						5	
Tasker *et al.*, 1980	12				6			19			69			

*: spontaneous death, killed at moribund condition or after abortion; a: reported maldosing; b: non-reported maldosing

Analysis of the Results in the Rabbit:
Incidence (%) of Dams with Loose Stools, Diarrhea, few or no Feces

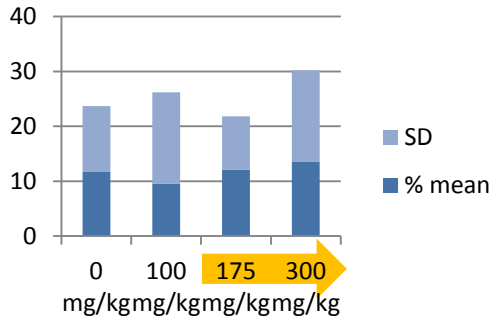
Study	Dose (mg/kg)													
	0	10	20	50	75	100	150	175	200	300	350	400	450	500
Coles and Doleman (1996)	0			6					13			62		
Moxon (1995)	22					25		58		100				
Hojo (1995)	0	0				0				24				
Suresh (1993)	0		0			6								80
Brooker *et al*. (1991)	?			+			++						+++	
Tasker *et al.*, 1980	+				+			++			+++			

+, ++, +++: increasing incidence of diarrhea and soft stools (no quantitative data reported)

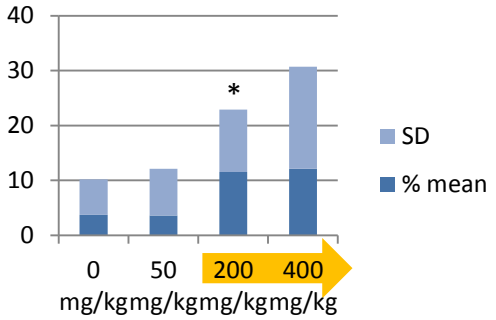
Analysis of the Results in the Rabbit:

Mean % Post-implantation loss

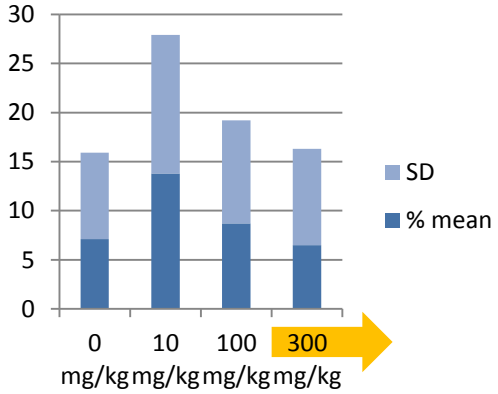
Moxon, 1996



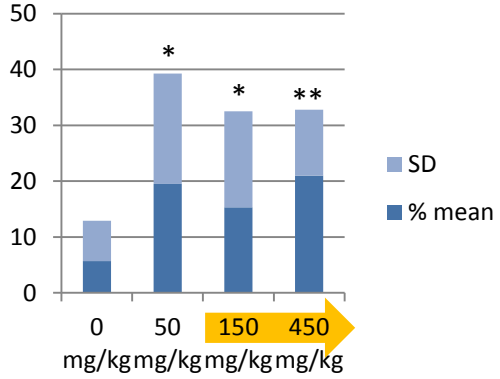
Coles and Doleman, 1996



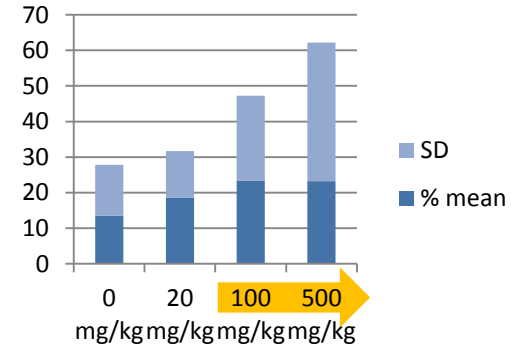
Hojo, 1995



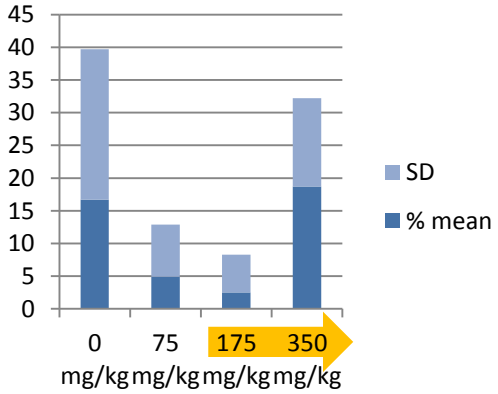
Brooker *et al*, 1991



Suresh, 1993

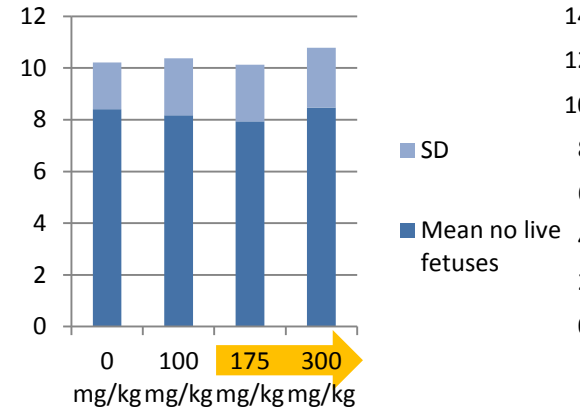


Tasker *et al*., 1980

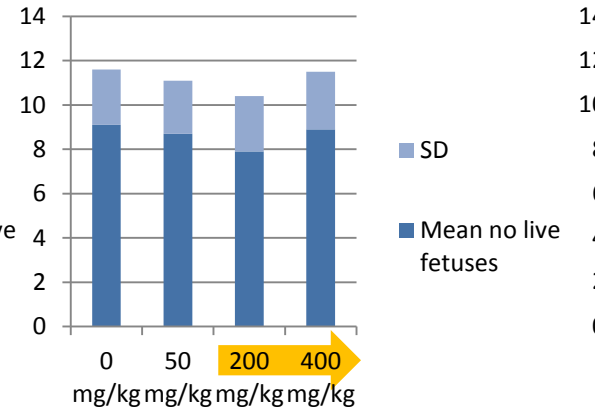


Analysis of the Results in the Rabbit:
Mean Number of Live Fetuses/Litter

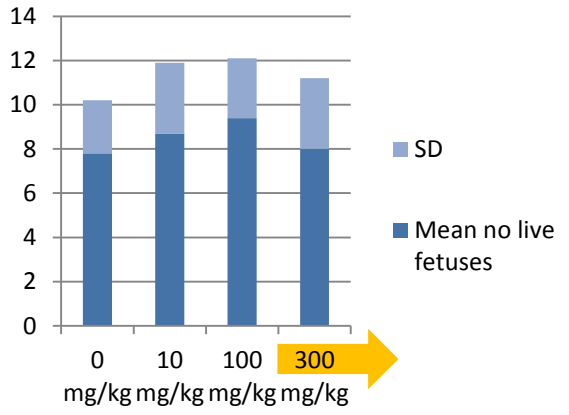
Moxon, 1996



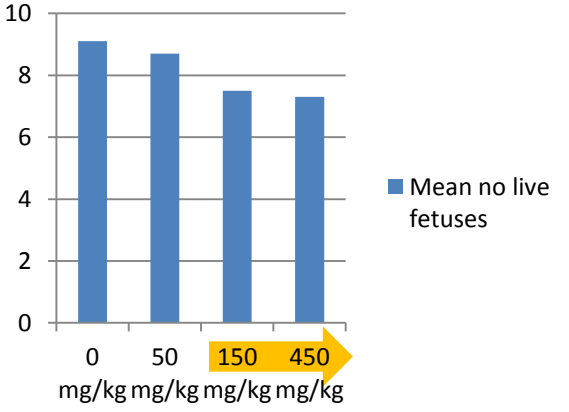
Coles and Doleman, 1996



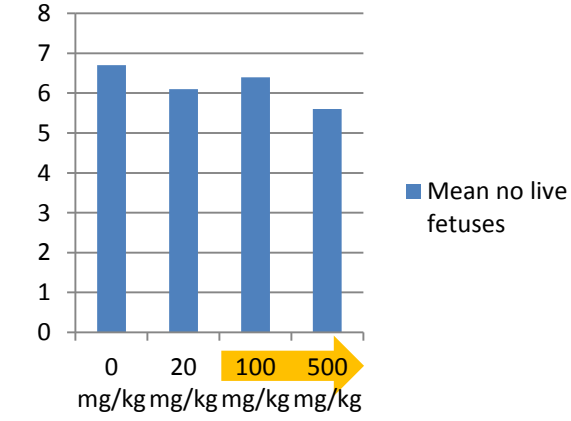
Hojo, 1995



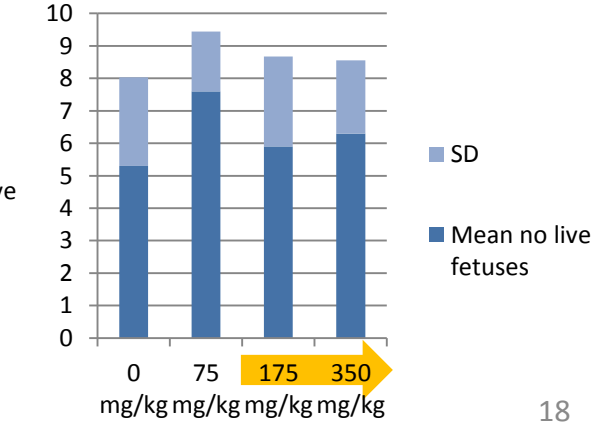
Brooker *et al*, 1991



Suresh, 1993

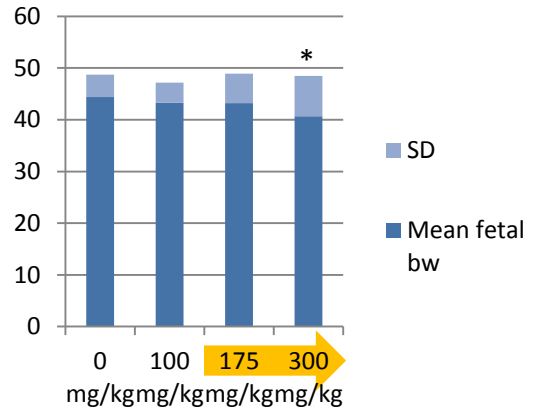


Tasker *et al*., 1980

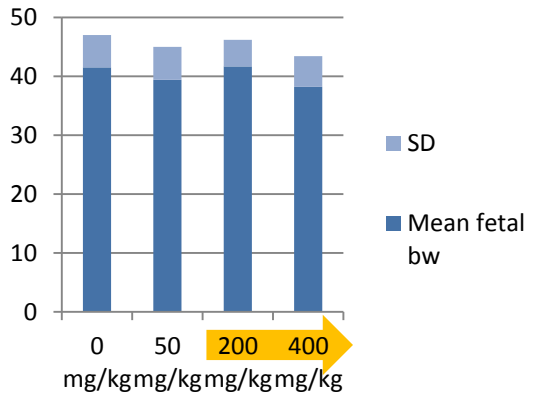


Analysis of the Results in the Rabbit:
Mean Fetal Body Weight (g)

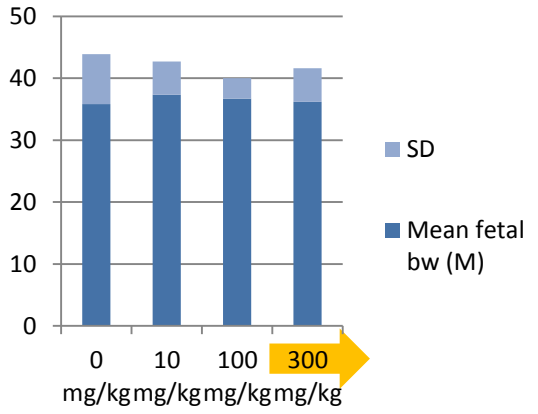
Moxon, 1996



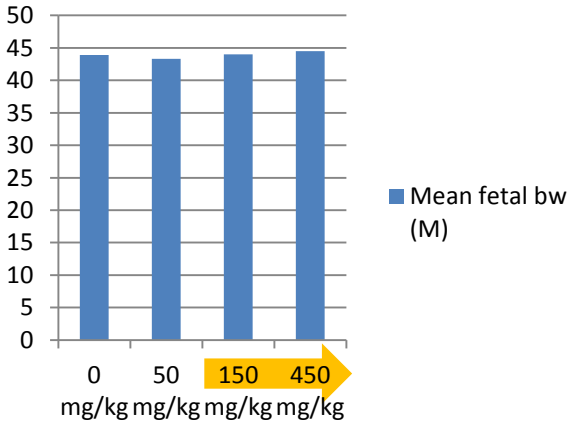
Coles and Doleman, 1996



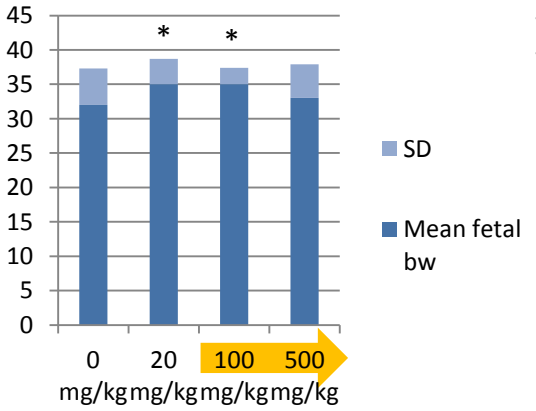
Hojo, 1995



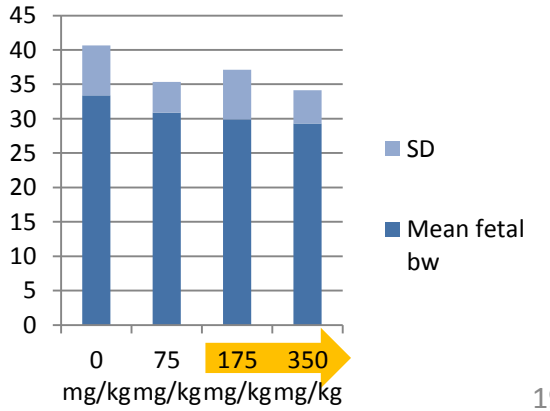
Brooker *et al*, 1991



Suresh, 1993



Tasker *et al*., 1980



Analysis of the Results in the Rabbit:
Total Malformations (Coles and Doleman, 1996)

Malformations: Total number of fetuses (litters)	Dose (mg/kg)			
	0	**100**	**175**	**300**
Number of fetuses examined	128	157	119	134
External/visceral and skeletal malformations	1(1)	3(2)	2(2)	1(1)
External/visceral and skeletal variations	41(13)	50(17)	39(15)	51(14)

Analysis of the Results in the Rabbit:
Total Malformations (Moxon, 1996)

Malformations/variations: Total number of fetuses (litters)	Dose (mg/kg)			
	0	**100**	**175**	**300**
Number of fetuses examined	143	147	135	144
External/visceral, major	2(2)	1(1)	0(0)	2(2)
External/visceral, minor	12(8)	7(5)	9(8)	11(7)
Skeletal, major	3(2)	0(0)	0(0)	1(1)
Skeletal, minor[a]	58 (16)	82(18)**	59(16)	79(17)**
External/visceral variations	0(0)	0(0)	0(0)	0(0)
Skeletal variations	119(17)	129(18)	116(17)	132(17)**

**: $p < 0.01$; a: reduced ossification of transverse processes of cervical and lumbar vertebrae, sternebrae and bones of the hindpaw

Analysis of the Results in the Rabbit:
Total Malformations (Hojo, 1995)

Malformations/variations: Total number of fetuses	Dose (mg/kg)			
	0	**10**	**100**	**300**
Number of fetuses examined	140	130	150	112
External malformations	0	0	2	0
Visceral malformations	0	1	3	0
Skeletal malformations	1	4	6	5
Visceral variations	4	5	5	1
Skeletal variations	40	32	61*	31

*: $p < 0.05$

Analysis of the Results in the Rabbit:
Skeletal Malformations (Hojo, 1995)

Skeletal malformations: Total number of fetuses	Dose (mg/kg)			
	0	**10**	**100**	**300**
Number of fetuses examined	140	130	150	112
Fusion of frontal/parietal bones	0	1	0	2
Fissure of parietal bone	0	0	3	0
Hypoplasia of interparietal bone	0	1	0	0
Splitting of parietal bones	0	0	3	1
Shortening of nasal/frontal/ mandibular bones	0	0	1	0
Hemivertebra	1	0	0	2
Unilateral ossification centre of thoracic/lumbar vertebral bodies	0	1	0	0
Bifurcation of ribs	1	0	0	0
Sternal cleft	0	0	1	0
Splitting of sternebrae with sternocostal joint displacement	0	2	0	0

Analysis of the Results in the Rabbit:
Total Malformations (Suresh, 1993)

Malformations/variations: Total fetuses (litters)	Dose (mg/kg)			
	0	**20**	**100**	**500**
Number of fetuses examined	133	79	77	28
External malformations	2(2)	2(1)	1(1)	0(0)
Visceral malformations	4(3)	6(3)	6(4)	8(2)*
Skeletal malformations	11(4)	5(3)	0(0)	1(1)
External variations	0(0)	0(0)	1(1)	0(0)
Visceral variations	(9)	(5)	(7)	(2)
Skeletal variations	(20)	(13)	(11)	(5)

* Statistically significantly different from control

Analysis of the Results in the Rabbit:
Total Malformations (Brooker *et al*., 1991)

Malformations/variations: Total number of fetuses (litters)	Dose (mg/kg)			
	0	**50**	**150**	**450**
Number of fetuses examined	163	104	112	95
External/visceral and skeletal malformations	3(3)	3(3)	5(3)	6(5)
External/visceral and skeletal variations	29(13)	26(9)	26(11)	16(10)

Analysis of the Results in the Rabbit:
Total Malformations (Tasker *et al*., 1980)

Malformations: Total number of fetuses (litters)	Dose (mg/kg)			
	0	**75**	**175**	**350**
Number of fetuses examined	63	114	65	38
External/visceral malformations	0(0)	0(0)	0(0)	2(1)
Skeletal malformations	0(0)	3(3)	2(2)	0(0)

Analysis of the Results in the Rabbit:

Cardio-vascular malformations, total number of fetuses(litters with malformations)

Study	Dose (mg/kg)													
	0	10	20	50	75	100	150	175	200	300	350	400	450	500
Coles and Doleman (1996)	0(0)			0(0)					1(1)			0(0)		
Moxon (1995)	1(1)					1(1)		0(0)		1(1)				
Hojo (1995)	0(0)	0(0)				1(1)				0(1)				
Suresh (1993)	2(2)		4(3)			6(4)								6(2)
Brooker et al. (1991)	1(1)			1(1)			4(3)						5(4)	
Tasker et al., 1980	0(0)				0(0)			0(0)			0(0)			

Analysis of the Results in the Rabbit:
Cardio-vascular Malformations (Suresh, 1993), Number of Fetuses (Litters with Malformations)

Malformations	Dose (mg/kg)			
	0	**20**	**100**	**500**
Number of fetuses examined	133	78	77	28
Seal-shaped heart	1(1)	0(0)	0(0)	0(0)
Cardiomegaly and seal-heart	0(0)	0(0)	1(1)	0(0)
Dilated heart	0(0)	4(3)*	4(2)*	5(2)*
Dilated ventricle (R)	0(0)	0(0)	0(0)	1(1)
Dilated ventricle (L)	0(0)	0(0)	1(1)	0(0)

* Statistically significantly different from control

Analysis of the Results in the Rabbit:
Thoracic Malformations (Brooker *et al*., 1991), Number of Fetuses (Litters with Malformations)

Malformations	Dose (mg/kg)			
	0	**50**	**150**	**450**
Number of fetuses examined	163	104	112	95
Right sided ascending aorta	0(0)	1(1)	0(0)	0(0)
Narrow/dilated aortic arch/pulmonary trunk/arterial trunk	1(1)	1(1)	1(1)	3(3)
Dorsally displaced pulmonary trunk	1(1)	0(0)	0(0)	0(0)
Retro-esophageal right subclavian artery	0(0)	0(0)	3(1)	2(1)
Single carotid artery	0(0)	1(1)	0(0)	0(0)
Inter-ventricular septal defect	1(1)	1(1)	1(1)	4(4)
Enlarged left, reduced right ventricles	0(0)	0(0)	0(0)	2(2)

Analysis of the Results in the Rabbit: Cardiovascular Malformations (Brooker *et al*., 1991), Incidence (%, fetal basis)

Malformations	Dose (mg/kg)				Historical control range*
	0	50	150	450	
Number of fetuses examined	163	104	112	95	5964
Narrow/dilated aortic arch/pulmonary trunk/arterial trunk	0.6	1	0.9	3.2	0-1.9** 0-1.7***
Retro-esophageal right subclavian artery	0	0	2.7[a]	2.1[b]	0-1.8
Inter-ventricular septal defect	0.6	1	0.9	4.2[c]	0-2.8
Enlarged left, reduced right ventricles	0	0	0	2.1[d]	0-1

*: 48 vehicle studies performed from January 1989 until October 1993 (Interfauna UK); **: dilated ascending aortic arch; ***: narrow ascending aortic arch; a: 3/112 vs 2/116-152 in the historical controls, and all malformations occurred in one litter; b: 2/95 vs 2/116-152 and all malformations occurred in one litter; c: 4/95 vs 3/106-152 in the historical controls; d: 2/95 vs 1/103-154 in the historical controls.

Discussion of the Results in the Rabbit:
Maternal Toxicity

- High pre-term mortality (spontaneous deaths, sacrifice because of moribund state or abortions) in the mid-and high dose groups of 2 studies (Suresh, 1993; Tasker et al., 1980). This invalidates the embryo-fetal developmental toxicity evaluation at these dose levels
- Mortality was due to malgavage, regurgitation of gastric content and gastro-intestinal intolerance to glyphosate acid
- Gastro-intestinal effects (observed in all studies) consisted of soft stools, diarrhea, few to no feces, stasis (hair balls), gastro-enteritis, watery fluid and gas in caecum and rectum. These GI effects lead to a decrease in food consumption and body weight
- The gastro-intestinal effects disturbed (interrupted) the night-day physiological cycle of the GI-tract of the rabbit whereby the animals were not anymore capable in consuming their soft fecal pellets at night. Soft fecal pellets are an important resource of nutrients for the (pregnant) rabbit

Discussion of the Results in the Rabbit:
Post-implantation Loss

- Statistically significant increase in % post-implantation loss at all dose levels (without a dose-effect relationship) in 1 study (Brooker et al., 1991). The % post-implantation loss at the low (19.5%) and high (21%) dose were slightly beyond the historical control range (3.6 – 17.5%). However, late embryonic deaths were also increased but remained within the historical control range whereas there was no dose-effect relationship for early embryonic death
- The % implantation loss in the control group (5.7%) was at the low end of the historical control range (3.6 – 17.5%)
- This result is not consistent with the outcome of all other studies where no effect on post-implantation loss was observed

Discussion of the Results in the Rabbit:
Fetal parameters

- No effect was noted on the mean number of fetuses per litter in all studies
- A statistically significant decrease was observed in mean fetal body weight in the high dose group in 1 study (Moxon, 1996)
- A statistically significant increase was observed in mean fetal body weight in the low and mid dose groups without a dose effect relationship in 1 study (Suresh, 1993)
- The decrease in mean fetal body weight at the high dose in the Moxon, 1996 study is an effect of retardation of fetal development due to the bad health condition of the dams at that dose level (decrease in food consumption, decrease in body weight, diarrhea, GI-effects)

Discussion of the Results in the Rabbit:
Malformations and Variations (1)

- In the Moxon, 1996 study a statistically significant increase in skeletal variations was observed in the high dose group. This effect can be ascribed to the bad health condition of the dams at that dose (300 mg/kg bw/day). There were no substance related effects on major and minor malformations
- In the Suresh, 1993 study a statistically significant increase was observed in visceral malformations in the high dose group (500 mg/kg bw/day) which is primarily due to an increased incidence of cardio-vascular malformations
- In the Hojo, 1995 study a statistically significant increase in skeletal variations was noted in the mid dose group without a dose-effect relationship. In the same study a non-statistically significant increase of skeletal malformations was observed at all dose levels but without a dose-effect relationship

Discussion of the Results in the Rabbit:
Malformations and Variations (2)

- In the Coles and Doleman, 1996 study no substance-related effects on malformations and variations were observed
- In the Brooker at al., 1991 study a non- statistically significant increase in malformations was observed in the mid and high dose groups. This increase is mainly due to the increase of cardio-vascular malformations
- In the Tasker, 1980 study a slight and non- statistically significant increase in external/visceral malformations was observed in the high dose group. There was no dose-related effect on skeletal malformations

Discussion of the Results in the Rabbit: Cardio-vascular Malformations (Suresh, 1993)

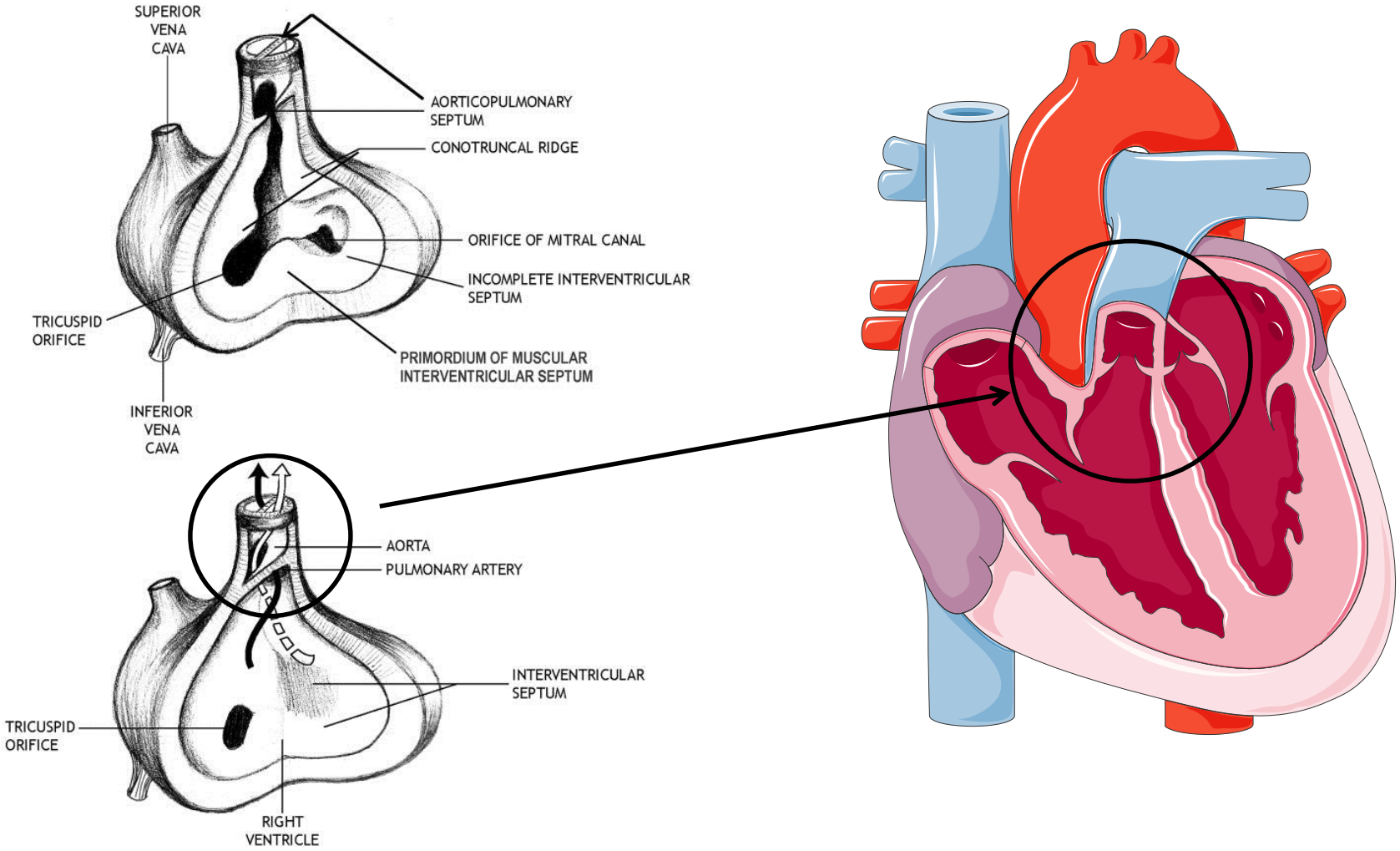
- The increase of cardio-vascular malformations in the Suresh, 1993 study is due to the "dilated heart" which was statistically significantly increased at all dose levels without a dose-effect relationship and was only observed in a limited number of litters per dose (2 to 3). No dose-effect relationship was found for the other cardio-vascular malformations in this study (seal-shaped heart, cardiomegaly, dilated ventricles)
- Dilated ventricles may occur if the rabbits are killed by methods that produce anoxia in the fetuses
- The statistically significant increase in the incidence of "dilated heart" in the Suresh, 1993 study is difficult to interpret since this unusual term is not defined in the study report and no criteria and measurements were given to allow comparison with the other studies
- These cardio-vascular effects have not been observed in the other studies as the effects seen in this study are not related to the cardio-vascular malformations in the Brooker et al., 1991 study

Discussion of the Results in the Rabbit: Cardio-vascular Malformations (Brooker *et al*., 1991)(1)

- The cardiovascular malformations reported in the Brooker et al., 1991 study occurred in the same animals and are related to a single morphogenetic mechanism i.e. displacement of the developing aortico-pulmonary septum which may adjust during the post-natal period. These mechanistically related findings often cluster together and include:
  - Narrow or dilated aorta and pulmonary artery
  - Inter-ventricular septal defect
  - Disproportionally sized right and left ventricles
- These findings were also observed (often in clusters) in the historical control data provided by the laboratory

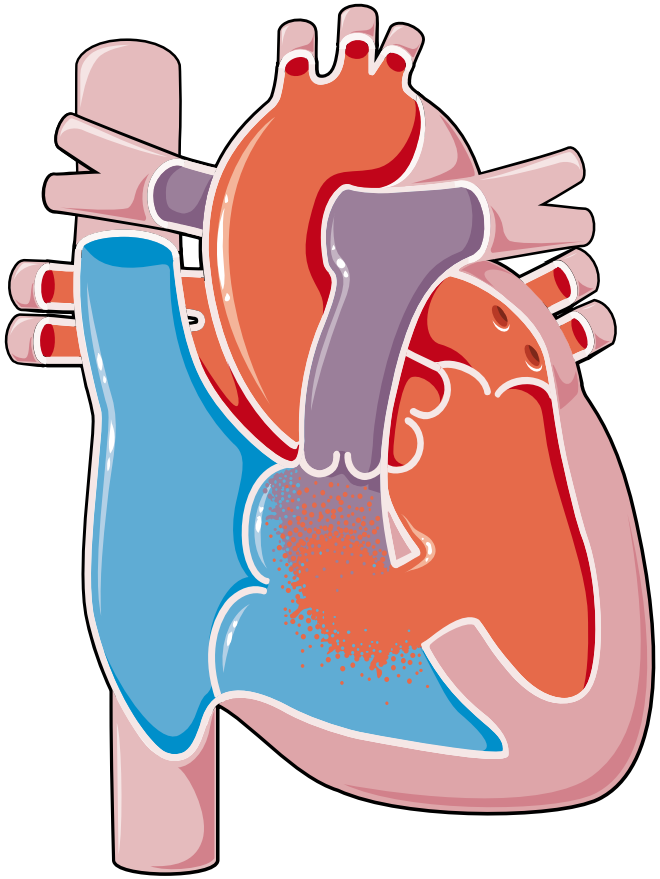
Discussion of the Results in the Rabbit:

Developing Aortico-pulmonary Septum



Discussion of the Results in the Rabbit:

Interventricular Septum Defect

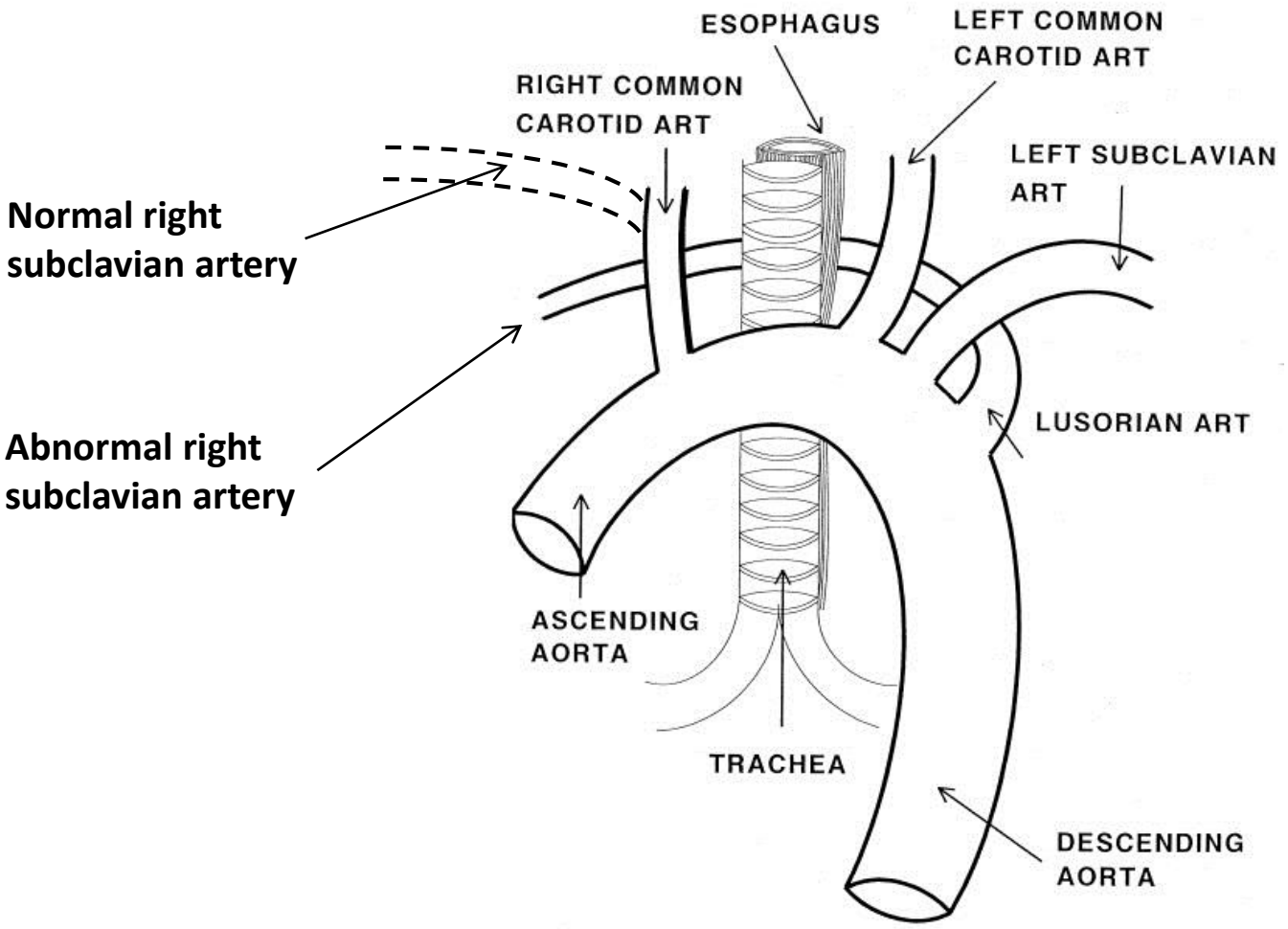


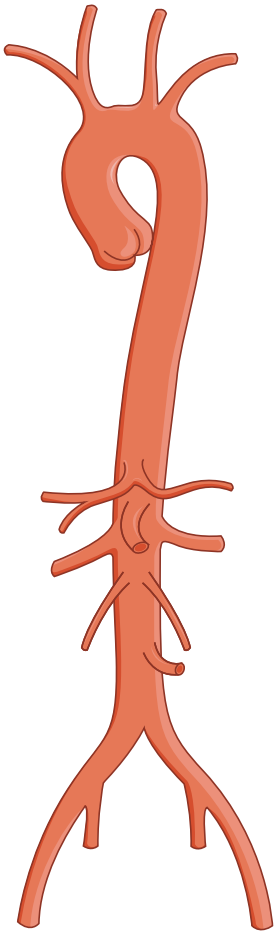
Discussion of the Results in the Rabbit: Cardio-vascular Malformations (Brooker *et al*., 1991)(2)

- Of the thoracic malformations observed in the Brooker et al., 1991 study only following malformations were increased with dose:
  - Narrow/dilated aortic arch/pulmonary trunk/arterial trunk (3.2% at high dose)
  - Retro-esophageal right subclavian artery (2.7% at mid dose and 2.1% at high dose, beyond the historical control range of 0-1.8%)
  - Inter-ventricular septal defect (4.2% at high dose, beyond the historical control range of 0-2.8%)
  - Enlarged left, reduced right ventricles (2.1% at high dose, beyond the historical control range of 0-1%)

Discussion of the Results in the Rabbit:

Retro-esophageal Right Subclavian Artery





Normal

Discussion of the Results in the Rabbit: Cardio-vascular Malformations (Brooker *et al*., 1991)(3)

- Although the incidences (in %, fetal basis) of these malformations were beyond the historical control range, there was only a very small difference in absolute terms: e.g. in the high dose group ventricular septum defects were observed in 4 fetuses (in 4 litters) whereas the maximum number of fetuses with this defect per control group in the historical controls was 3
- The retro-esophageal right subclavian artery is a malformation that is not related to the morphogenetic mechanism involving the formation of the spiral septum and is not uncommon. In man, this condition is found in 0.5 to 2.0% of subjects
- There is an inconsistent pattern of the most commonly occurring cardiac defects without a clear dose-related effect
- The increased incidence with dose in cardio-vascular malformations as observed in the Brooker et al., 1991 study was not confirmed in the other studies in the rabbit

Overall Conclusion

Conclusion of the Results in the Rabbit

- There is no relationship between the cardio-vascular malformations noted in the Suresh, 1993 study ("dilated heart") and those observed in the Brooker *et al*., 1991 study (defect in the development of the aortico-pulmonary trunk) and these effects were not confirmed in the other rabbit studies
- The adverse developmental effects in the rabbit (increased post implantation loss (1 study), reduction of fetal weight (in 1 study) and cardio-vascular malformations (in 2 studies but with unrelated malformations) only occurred at dose levels with severe maternal toxicity

Classification

Classification as Toxic to Reproduction:
Embryo-fetal Development (1)

- Category 1A: Known human reproductive toxicant
- Category 1B: Presumed human reproductive toxicant largely based on data from animal studies
  - Clear evidence of an adverse effect on development in the absence of other toxic effects, or
  - The adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects
- Category 2: Suspected human reproductive toxicant
  - Some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development, and
  - The evidence is not sufficiently convincing to place the substance in Category 1 (deficiencies in the study)
  - The adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

Classification as Toxic to Reproduction:
Embryo-fetal Development (2)

- No effects on embryo-fetal development have been observed in the rat (6 studies of which 4 are GLP and TG compliant)
- The delayed ossification and reduced fetal body weight in the rabbit (Moxon, 1996) are clearly the consequence of the severe maternal toxicity at the high dose
- The increased post-implantation loss (Brooker *et al*., 1991) and the cardio-vascular malformations (dilated heart in the Suresh, 1993 study and inter-ventricular septum defects in the Brooker *et al*., 1991 study are not related) have not been confirmed in the other studies and only occurred in the presence of severe maternal toxicity
- **The weight-of-evidence analysis based on the results of all rat and rabbit studies indicates that Glyphosate does not produce embryo-fetal developmental toxicity, therefore Glyphosate should not be classified as toxic to reproduction**

Back-up Slides

OECD test Guideline 414:
Test Conditions

- The preferred species are the rat and the rabbit. Another species can be used when this can be justified (e.g. the mouse in the case of gastro-intestinal intolerance in the rabbit)
- Approximately 20 female animals with implantation sites at necropsy per dose group
- Dose groups with fewer than 16 animals may be inappropriate
- Maternal mortality should not exceed 10%
- Daily administration from implantation (e.g. day 5 post-mating to the day prior to scheduled sacrifice or at the end of organogenesis)
- At least 3 dose groups and 1 control group
- The highest dose should induce some developmental and/or maternal toxicity. The lowest dose shouldn't produce any effect
- The limit dose is 1000 mg/kg bw/day

OECD test Guideline 414:
Observations

- Clinical signs
- Body weight and food consumption
- Gross necropsy of the dams
- Weight of gravid uteri including cervix
- Number of corpora lutea
- Number of embryonic or fetal deaths
- Number of viable fetuses
- Body weight and sex of each fetus
- External alterations
- Visceral alterations
- Skeletal alterations

Analysis of the Results in the Rabbit:

Embryonic Death and Post-implantation loss (Brooker *et al*., 1991)

Malformations	Dose (mg/kg)				Historical control range
	0	50	150	450	
Number of litters	18	12	15	13	
Early embryonic deaths/litter	0.4	0.9	0.9	0.5	0.3-1.1
Late embryonic deaths/litter	0.2	0.9	0.5	1.3**	0.1-1.3
Total embryonic deaths/litter	0.6	1.8*	1.5*	1.8**	0.6-2.0
Post implantation loss (%)	5.7	19.5*	15.3*	21**	3.6-17.5[a]

*: <0.05; **: p<0.01; a: 48 vehicle studies performed from January 1989 until October 1993 (Interfauna UK)

Analysis of the Results in the Rabbit:

Cardio-vascular Malformations (Brooker *et al*., 1991), Number of Fetuses

Malformations	Dose (mg/kg)			
	0	**50**	**150**	**450**
Number of fetuses examined	163	104	112	95
Narrow ascending aorta Dorsally displaced pulmonary trunk IV septal defect	1	0	1	1
Dilated ascending aorta/aortic arch Narrow pulmonary trunk IV septal defect with enlarged left, reduced right ventricle	0	0	0	2
Retro-esophagial right subclavian artery IV septal defect (1 fetus at high dose)	0	0	3	2
Single dilated arterial trunk and carotid artery Right-sided descending aorta IV septal defect	0	1	0	0