Repeated Dose Specific Target Organ Toxicity Classification of Glyphosate

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Toxicology Studies

- In this presentation:
  - Only consideration is given to the results of the most recent oral repeated dose toxicity studies that have been performed with technical glyphosate (glyphosate acid)
  - Results of oral repeated dose toxicity studies of 90 days and longer are considered that are in compliance with internationally agreed test guidelines (OECD TG 408, 409, 451, 452, 453) applicable at the time of conduct of the study and that are performed in accordance with good laboratory practices (GLP)
  - By way of exception also embryo-fetal development toxicity studies in the rabbit (TG 414) are considered for the evaluation of repeated dose toxicity
  - 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 Rat Data

Repeated Dose Toxicity Studies of Glyphosate in the Rat

Study	*Strain*	*Duration of treatment (months)*	*Doses (mg/kg bw/day)*	*Comment*
Botham (1996)	Wistar derived, Alpk:APfSD	3	M: 0-81-414-1612 F: 0-90-447-1821	
Coles *et al*. (1996)	Sprague-Dawley, CD	3	M: 0-79-730-3706 F: 0-90-844-4188	
Kinoshita (1995)	Sprague-Dawley, Crj:CD	3	M: 0-168-569-1735 F: 0-195-637-1892	
Milburn (1996)	Wistar derived, Alpk:APfSD	12	M: 0-141-560-1409 F: 0-167-671-1664	
Wood (2009)	Wistar Han, Crl: WI	24	M: 0-86-285-1077 F: 0-105-349-1382	
Brammer (2001)	Wistar derived, Alpk:APfSD	24	M: 0-121-361-1214 F: 0-145-437-1498	
Enomoto (1997)	Sprague-Dawley Crj:CD	24	M: 0-104-354-1127 F: 0-115-393-1247	

Bhide (1997) study was not considered here because not found acceptable by RMS

Repeated Dose Toxicity Studies of Glyphosate in the Rat:

Results

Study	*Duration of treatment (months)*	*NOAEL (mg/kg bw/day)*	*LOAEL (mg/kg bw/day)*	*Effects at LOAEL*
Botham (1996)	3	M: 414 F: 447	M: 1612 F: 1821	Reduced body weight gain in males
Coles *et al*. (1996)	3	M: 79 F: 90	M: 730 F: 844	Flattening of the intestinal mucosa (stretch atrophy due to caecal distention)
Kinoshita (1995)	3	M: 168 F: 195	M: 569 F: 637	Distention of the caecum in males
Milburn (1996)	12	M: 141 F: 167	M: 560 F: 671	Decrease in food consumption, decrease in body weight, basophilia of acinar cells of the parotid salivary gland in females
Wood (2009)	24	M: 285 F: 349	M: 1077 F: 1380	Decrease in body weight gain in males, fatty infiltration of the bone marrow
Brammer (2001)	24	M: 361 F: 437	M: 1214 F: 1498	Slight increase in proliferative cholangitis in the liver and papillary necrosis in the kidney. Increase in hepatitis and prostatitis
Enomoto (1997)	24	M: 104 F: 115	M: 354 F: 393	Decrease in body weight gain, distention of the caecum, follicular hyperkeratosis

Repeated Dose Toxicity Studies of Glyphosate in the Rat:
Conclusions (1)

- The NOAELs that can be reasonably derived from the rat studies are:
  - 168 mg/kg bw/day (males) and 195 mg/kg bw/day (females) for an exposure during 3 months
  - 141 mg/kg bw/day (males) and 167 mg/kg bw/day (females) for an exposure during 12 months
  - 104 mg/kg bw/day (males) and 115 mg/kg bw/day (females) for an exposure during 24 months

Repeated Dose Toxicity Studies of Glyphosate in the Rat:
Conclusions (2)

- The LOAELs that can be reasonably derived from the rat studies are:
  - 569 mg/kg bw/day (males) and 637 mg/kg bw/day (females) for an exposure during 3 months
  - 560 mg/kg bw/day (males) and 671 mg/kg bw/day (females) for an exposure during 12 months
  - 354 mg/kg bw/day (males) and 393 mg/kg bw/day (females) for an exposure during 24 months

Repeated Dose Toxicity Studies of Glyphosate in the Rat:
Conclusions (3)

- No specific target organs could be identified in a consistent way
- Some effects such as papillary necrosis in the kidneys or basophilia of the acinar cells in the salivary gland, hepatitis and prostatitis were noted in one single study but were not confirmed in the other studies
- Decrease in body weight gain and distention of the caecum were observed in several studies

Analysis of Mouse Data

Repeated Dose Toxicity Studies of Glyphosate in the Mouse

Study	*Strain*	*Duration of treatment (months)*	*Doses (mg/kg bw/day)*	*Comment*
Kuwahara (1995)	CD-1, Crj:CD-1	3	M: 0-600-1221-6295 F: 0-765-1486-7435	
Wood *et al*. (2009)	CD-1, Crl:CD-1 (ICR) BR	18	M: 0-71-234-810 F: 0-98-300-1081	
Kumar (2001)	Swiss albino, HsdOla: MF1	18	M: 0-15-150-1453 F: 0-15-151-1467	
Sugimoto (1997)	CD-1, SPF ICR, Crj:CD-1	18	M: 0-165-838-4348 F: 0-153-787-4116	

Repeated Dose Toxicity Studies of Glyphosate in the Mouse:

Results

Study	*Duration of treatment (months)*	*NOAEL (mg/kg bw/day)*	*LOAEL (mg/kg bw/day)*	*Effects at LOAEL*
Kuwahara (1995)	3	M: 1221 F: 1486	M: 6295 F: 7435	Cystitis of the urinary bladder, distention of the caecum
Wood *et al*. (2009)	18	M: 810 F: 1081	M: > 810 F: > 1081	No treatment related effects
Kumar (2001)	18	M: 150 F: 151	M: 1453 F: 1467	Increased incidence in malignant lymphoma
Sugimoto (1997)	18	M: 838 F: 153	M: 4348 F: 787	Decrease in food consumption and body weight gain in females

Repeated Dose Toxicity Studies of Glyphosate in the Mouse:
Conclusions (1)

- The NOAELs that can be reasonably derived from the mouse studies (non-neoplastic pathology) are:
  - 1221 mg/kg bw/day (males) and 1486 mg/kg bw/day (females) for an exposure during 3 months
  - 838 mg/kg bw/day (males) and 153 mg/kg bw/day (females) for an exposure during 18 months

Repeated Dose Toxicity Studies of Glyphosate in the Mouse:
Conclusions (3)

- No specific target organs could be identified in a consistent way
- Some effects such as cystitis of the urinary bladder and distention of the caecum were reported in one study but not confirmed in the other studies
- Decrease in food consumption and body weight gain were reported in one study

Analysis of Dog Data

Repeated Dose Toxicity Studies of Glyphosate in the Dog

Study	*Strain*	*Duration of treatment (months)*	*Doses (mg/kg bw/day)*	*Comment*
Gaou (2007)	Beagle	3	0-30-300-1000	capsules
Prakash (1999)	Beagle	3	M: 0-5.2-54-252 F: 0-5.4-53-253	diet
Yoshida (1996)	Beagle	3	M: 0-40-198-1015 F: 0-40-201-1014	diet
Hodge (1996)	Beagle	3	M: 0-68-323-1680 F: 0-68-334-1750	Diet, test material of very high purity (99.1 w/w)
Haag (2008)	Beagle	12	0-30-125-500	Capsules
Nakashima (1997)	Beagle	12	M: 0-34-182-1203 F: 0-37-184-1259	Diet, uncertainty about dose levels actually tested
Brammer (1996)	Beagle	12	M: 0-91-440-907 F: 0-91-448-926	Diet

Repeated Dose Toxicity Studies of Glyphosate in the Dog:

Results

Study	*Duration of treatment (months)*	*NOAEL (mg/kg bw/day)*	*LOAEL (mg/kg bw/day)*	*Effects at LOAEL*
Gaou (2007)	3	300	1000	Poor clinical condition (liquid or soft feces, vomiting, thin appearance, decrease in body weight, dehydration)
Prakash (1999)	3	M: 252 F: 253	M: > 252 F: >253	No treatment related effects
Yoshida (1996)	3	M: 1015 F: 1014	M: > 1015 F: > 1014	No treatment related effects
Hodge (1996)	3	M: 323 F: 334	M: 1680 F: 1750	Slightly reduced body weight gain in females
Haag (2008)	12	500	> 500	No treatment related effects
Nakashima (1997)	12	M: 182 F: 184	M: 1203 F: 1259	Retarded body weight gain and loose stools in males and females. Decrease in RBC parameters in females
Brammer (1996)	12	M: 440 F: 448	M: 907 F: 926	Decrease in body weight gain in females

Repeated Dose Toxicity Studies of Glyphosate in the Dog:
Conclusions (1)

- The NOAELs that can be reasonably derived from the dog studies are:
  - 323 mg/kg bw/day (males) and 334 mg/kg bw/day (females) for an exposure during 3 months
  - 440 mg/kg bw/day (males) and 448 mg/kg bw/day (females) for an exposure during 12 months

Repeated Dose Toxicity Studies of Glyphosate in the Dog:
Conclusions (2)

- The LOAELs that can be reasonably derived from the dog studies are:
  - 1000 mg/kg bw/day (males and females) for an exposure during 3 months
  - 907 mg/kg bw/day (males) and 926 mg/kg bw/day (females) for an exposure during 12 months

Repeated Dose Toxicity Studies of Glyphosate in the Dog:
Conclusions (3)

- No specific target organs could be identified in a consistent way
- The decrease in RBC parameters observed in one study was not confirmed in the other studies
- The poor clinical condition and the decrease in body weight gain were reported in several studies

Analysis of Rabbit Data

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 mal-dosing; b: non-reported maldosing

The rabbit studies that showed an increase in pre-term mortality (either found dead or killed because of abortion or moribund) at dose levels up to (and including) 300 mg/kg bw/day are Coles and Dolman, 1996; Moxon, 1996; Hojo, 1995; Suresh, 1993 and Tasker *et al*., 1980.

Analysis of the Results in the Rabbit:
Mortalities at doses up 300 mg/kg bw/day (Coles and Doleman, 1996)

Dose (mg/kg bw/day)	*Mortality*	*Comments*
0	1/18 (found dead)	1 rabbit died at 2 min after dosing, blood in thorax, inflated appearance of lungs, large area of congestion of right caudal lobe of the lung (malgavage)
200	1/18 (found dead)	1 rabbit died at GD16, red lungs, fluid filled thorax, test material in thoracic cavity (malgavage)

Repeated Dose Toxicity Studies of Glyphosate in the Rabbit:
Discussion Coles and Doleman, 1996 study

- From all the rabbit studies considered the Coles and Doleman, 1996 study can be dismissed because the 2 animals found dead (one at 0 and one at 200 mg/kg bw/day) had lung lesions (blood in thorax, inflated lungs, red lungs, fluid filled thorax, …) that are indicative of malgavage
- At the high dose level (400 mg/kg bw/day), one female was found dead (GD19) and one female was killed (GD20) for humane reasons. Clinical observations of these animals were hunched posture, lethargy, ptosis, hypothermia and blood on the litter tray
- At the high dose level, 10/16 animals suffered from diarrhea and 2/16 from reduced fecal output

Analysis of the Results in the Rabbit:
Mortalities at doses up 300 mg/kg bw/day (Moxon, 1995)

Dose (mg/kg bw/day)	*Mortality*	*Comments*
0	1/20 (killed)	Rabbit 20: aborted at GD30, loss of body weight, decrease in food consumption, diarrhea (GD23-30), mucus in faeces, few faeces (GD22-30)
100	2/20 (killed)	Rabbit 28: aborted at GD19, loss of body weight, decrease in food consumption, few faeces (GD10-19), diarrhea (GD12-17) Rabbit 40: aborted at GD25, loss of body weight, decrease in food conumption, few faeces (GD13-17), no faeces (GD19-25), diarrhea (GD14-19)
175	2/20 (killed)	Rabbit 49: killed for humane reasons, loss of body weight, decrease in food consumption, thin and subdued, few faeces (GD8-17), no faeces (GD17-23), diarrhea (GD14-18) Rabbit 58: aborted at GD22, loss of body weight, decrease in food consumption, few faeces (GD4-7, GD14-18, GD 21-22), diarrhea (GD14-22)
300	2/20 (killed)	Rabbit 62: aborted at GD24, loss of body weight, decrease in food consumption, few faeces (GD15-23), no faeces (GD18-24), diarrhea (GD12-23) Rabbit 72: aborted at GD23, loss of weight, decrease in food consumption, few faeces (GD14-23), diarrhea (GD14-23)

Repeated Dose Toxicity Studies of Glyphosate in the Rabbit:
Discussion Moxon, 1996 study (1)

- This study is by far the best reported study allowing the tracing down of the cause of death of all animals that died during the study before end of term. This study did not indicate any dose-effect relationship in the rabbits that needed to be killed either for humane reasons or for abortion. Only in the control group there was one rabbit that needed to be sacrificed
- All rabbits in this study that had to be killed (mostly for abortion) suffered from loss of body weight, decrease in food consumption, diarrhea, few or no fecal pellets, brown to green to red/black watery content of the caecum, gas formation in the caecum
- Accumulation of hair-like substance (hair balls) in the stomach is indicative of gastro-intestinal stasis

Analysis of the Results in the Rabbit:
Mortalities at doses up 300 mg/kg bw/day (Hojo, 1995)

Dose (mg/kg bw/day)	Mortality	Comments
300	1/17 (found dead)	Died at GD20, cause of death not known
	2/17 (killed)	1 rabbit aborted 1 rabbit with premature delivery,

Repeated Dose Toxicity Studies of Glyphosate in the Rabbit:

Discussion Hojo, 1995 study

- At 300 mg/kg bw/day, one rabbit died (cause of death not reported) and 2 had to be killed because of abortion. Both rabbits had a hair bolus in the stomach and watery content in the large intestine and caecum
- The hair bolus in the stomach is indicative of the slowing down (or arrest) of gastric emptying into the small intestine
- The rabbits aborted because of the deficiency of the gastro-intestinal system to maintain the cyclic (day-and-night cycle) physiology, necessary to maintain the nutritional status of the dam and the uterine content
- Gastro-intestinal disturbance causes also stress to the dams which can be contributory to abortion which is a well known phenomenon in rabbit embryo-fetal developmental toxicity studies

Analysis of the Results in the Rabbit:
Mortalities at doses up 300 mg/kg bw/day (Suresh, 1993*)

Dose (mg/kg bw/day)	Mortality	Comments
0	2/26 (found dead)	Rabbit 23 died at GD7, aspiration of gavage fluid? Rabbit 26 died at GD21, rales (GD16), esophageal injury (mal-gavage)
100	4/16 (found dead)	Rabbit 1: died at GD7, lung and trachea congestion, froth in trachea Rabbit 7: died at GD18, soft stool/diarrhea (GD7), caecum enteritis Rabbit 8: died at GD7, no signs reported at necropsy Rabbit 13: died at GD9, no signs reported at necropsy

*: Considered supplementary because of the low pregnancy rate at all dose levels, high mortality (25 and 53% at 100 and 500 mg/kg bw/day, respectively) and reporting deficiencies.

Repeated Dose Toxicity Studies of Glyphosate in the Rabbit:

Discussion Suresh, 1993 study

- 2 deaths in the control group, 1 death in the mid dose (100 mg/kg bw/day) group and 5 out of the 8 deaths reported in the high dose group (500 mg/kg bw/day) showed clinical signs and/or esophageal, tracheal and/or pulmonary injuries (e.g. rales, trachea congestion, lung congestion, lung emphysema, collapsed lungs) that can be attributed to malgavage
- Bad gavage practices combined with the poor reporting of the results put the reliability of this study in question

Analysis of the Results in the Rabbit:
Mortalities at doses up 300 mg/kg bw/day (Tasker *et al*., 1980*)

Dose (mg/kg bw/day)	Mortality	Comments
0	2/16 (killed)	2 rabbits aborted at GD22, soft stools/diarrhea
75	1/16 (found dead)	1 rabbit found dead at GD26, pneumonia, soft stools/diarrhea
175	1/16 (killed)	1 rabbit aborted at GD27, soft stools/diarrhea
	2/16 (found dead)	1 rabbit found dead at GD22, soft stools/diarrhea, gastro-enteritis 1 rabbit died at GD25, soft stools/diarrhea, no specific observations at necropsy

*: Considered supplementary because of too high mortality (69%) at the high dose level (350 mg/kg bw/day)

Repeated Dose Toxicity Studies of Glyphosate in the Rabbit:

Discussion Tasker *et al*., 1980 study

- This study can be dismissed because animals from all groups (including the control group) suffered from soft stools and diarrhea indicating that there must have been a problem with the rabbit feed (too low fiber content?) causing gastro-intestinal disturbance
- The increased incidence in pre-term mortality (69%) at the high dose (350 mg/kg bw/day) can be attributed to the effect of glyphosate acid on the functioning of the gastro-intestinal tract of the rabbit

Repeated Dose Toxicity Studies of Glyphosate in the Rabbit:

Effect of Glyphosate on the Functioning of the GI-tract (1)

- Gastro-intestinal stasis (hair accumulation in the stomach), water retention in the caecum and gas formation in the caecum and rectum are indicative of a complete abolishment of the unique and sensitive gastro-intestinal physiology of the rabbit (day-and-night cycle)
- The watery content of the caecum suggests the decrease of water re-absorption from the colon combined with the extraction of water from the circulatory system into the gastro-intestinal tract through osmosis caused by the presence of large amounts of unabsorbed glyphosate. This condition can lead to hemo-concentration, hypovolemia and shock
- Diarrhea and loose stools prevent the rabbit from eating the nutritional soft fecal pellets at night with a loss of weight, starvation and abortion as a consequence

Repeated Dose Toxicity Studies of Glyphosate in the Rabbit:
Conclusion (1)

- The investigation of the cause of death in the embryo-fetal development studies in the rabbit has indicated that the mortalities are due to either:
  - Malgavage with spilling of glyphosate acid in the respiratory tract causing aspiration pneumonia and irritation
  - Entry of glyphosate in the respiratory tract through regurgitation of gastric content causing irritation due to the acidity of the gastric content
  - Abolishment of the gastro-intestinal physiological cycle by glyphosate (irritation, osmosis, large amounts of unabsorbed material) with diarrhea preventing rabbits from eating the soft fecal pellets leading to malnutrition, loss of body weight and abortion

Repeated Dose Toxicity Studies of Glyphosate in the Rabbit:
Conclusion (2)

- All these effects are related to the local activity of glyphosate acid in the gastro-intestinal tract of the rabbit **which is unique and of no relevance to humans**
- When rabbits are exposed to glyphosate for 21 to 28 days via the dermal route no systemic toxicity or mortality is observed up to dose levels of 5000 mg/kg bw/day (Tornai, 1994; Johnson, 1982; Bhide, 1985)
- The dermal dose of 5000 mg/kg bw/day corresponds with an oral dose of 665 mg/kg bw/day when a dermal absorption rate of 2.66% (Hadfield, 2012) and an oral bio-availability of 20% (Bfr, 2015) are taken into consideration
- **In conclusion, glyphosate does not produce specific target organ toxicity in rabbits after repeated exposure apart from the local disturbance of the functioning of the GIT which is of no relevance to human health**

Classification

CLP Classification:

Specific Target Organ Toxicity after Repeated Dosing via the Oral Route of Exposure (1)

- **Category 1:** Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of:
  - Reliable and good quality evidence from human cases or epidemiological studies, or
  - Observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, **of relevance to human health**, were produced at generally low exposure concentrations. Guidance dose/concentration values are to be used as part of a **weight-of- evidence evaluation**.

CLP Classification:

Specific Target Organ Toxicity after Repeated Dosing via the Oral Route of Exposure (2)

- **Category 2:** Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, **of relevance to human health**, were produced at generally moderate exposure concentrations. In exceptional cases human evidence can also be used to place a substance in this Category

CLP Classification:

Specific Target Organ Toxicity after Repeated Dosing via the Oral Route of Exposure (3)

Category	*Equivalent guidance values (mg/kg bw/day)*	
	28-day	**90-day**
1	≤ 30	≤ 10
2	≤ 300	≤ 100

The guidance values refer to effects seen in a standard 90-day toxicity study conducted in rats. They can be used as a basis to extrapolate equivalent guidance values for toxicity studies of greater or lesser duration, using dose/exposure time extrapolation similar to Haber's rule for inhalation, which states essentially that the effective dose is directly proportional to the exposure concentration and the duration of exposure. The assessment shall be done on a case-by-case basis. For a 28-day study the guidance values are increased by a factor of three

CLP Classification:
STOT-RE Classification of Glyphosate

- The LOAEL values of glyphosate in the rat, the mouse and the dog treated orally for 90 days or longer are all greater than the category 2 guidance value for 90-day studies i.e. 100 mg/kg bw/day
- Mortality occurred in the rabbit (treated orally for about 2 weeks) at dose levels lower than the category 2 guidance value for 28-day studies i.e. 300 mg/kg bw/day, but the cause of death was attributed either to malgavage or/and intolerance of the gastro-intestinal system of the rabbit for glyphosate acid. The cyclic physiology of the **gastro-intestinal system of the rabbit is very sensitive and unique and not relevant to man**
- Since "Substance-induced species-specific mechanisms of toxicity, i.e. **demonstrated with reasonable certainty to be not relevant for human health, shall not justify classification**" the rabbit should not be used as a basis for STOT-RE classification
- **Therefore, glyphosate should not be classified for specific target organ toxicity**

Back-up Slides

OECD test Guideline 408 (Rodents):
Test Conditions

- The preferred species is the rat but also the mouse can be used
- At least 10 animals per sex should be used per dose group
- At least 3 dose groups and 1 control group should be used except where a limit test is conducted
- The test substance should be administered daily seven days per week for a period of 90 days. Administration for 5 days per week is allowed it that can be justified.
- The highest dose should induce toxicity but no death or severe suffering
- The limit dose is 1000 mg/kg bw/day

OECD test Guideline 408 (Rodents):
Observations

- General clinical signs
- Detailed clinical signs
- Neuro-behavioral signs
- Ophthalmology
- Body weight
- Food/water consumption
- Hematology
- Blood biochemistry
- Urinalysis (optional)
- Gross necropsy
- Histopathology

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 (Maternal Toxicity)

- Clinical signs
- Body weight and food consumption
- Gross necropsy of the dams, but:
  - No neuro-behavioral observations
  - No ophthalmology
  - No hematology
  - No blood biochemistry
  - No full macroscopic pathology
  - No histopathology

Analysis of the Results in the Rabbit:

Incidence (%) of Dams with Loose Stools, Diarrhea, few or no Feces

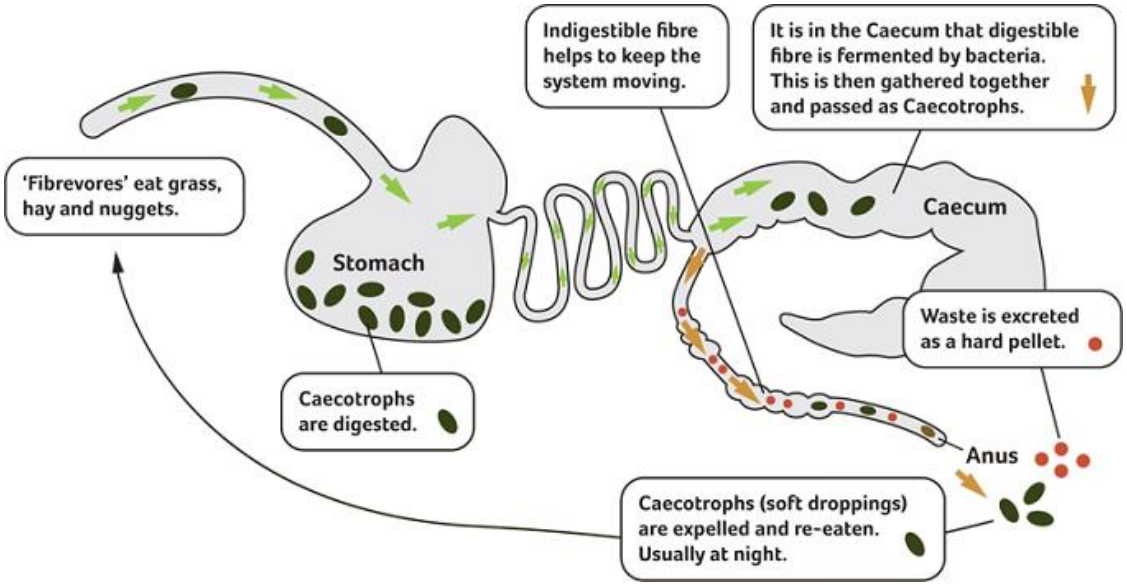
Study	Dose (mg/kg)													
	0	10	20	50	75	100	150	175	200	300	350	400	450	500
Coles and Doleman (1996)	0			6					13			62		
Moxon (1995)	22					25		58		100				
Hojo (1995)	0	0				0				24				
Suresh (1993)	0		0			6								80
Brooker *et al*. (1991)	?			+			++						+++	
Tasker *et al*., 1980	+				+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

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

The GIT Physiology of the Rabbit (1)

- The rabbit deviates from other laboratory animals as it is a herbivore, or more specifically a folivore, designed to exist on a diet of succulent green vegetation. Therefore, the GIT of the rabbit has evolved to a cyclic digestive system that is radically different from rats or mice and from larger herbivores
- Two types of feces are produced: the firm and dry daytime fecal pellets and the soft, moist night time fecal pellets (caecotrophs). The latter type of feces, that are directly ingested by the rabbits from the anus, are covered with mucus to protect them from the acid environment of the stomach and have a high content of water, nitrogen, electrolytes, vitamin B and other vitamins
- The rabbit's GIT physiology has therefore evolved into a complex and vulnerable system that centers around bacterial fermentation in the caecum and separation of digestible and indigestible components of the diet in the proximal colon
- To maintain the motility of the caecum and the colon a consistently high fibre diet is needed. Most of the common GIT problems seen in captive rabbits are related to inappropriate diets and infrequent feeding of treats to which the rabbit is not accustomed

The GIT Physiology of the Rabbit (2)



Stomach pH is much lower in rabbits (1.9) than in other animal species such as rat (3.3-5.0), mouse (3.3-4.5), dog (3.4-5.5) and monkey (2.8-4.8). The small intestine is relatively short making up approximately 12% of the total length of the GIT. The more extensive large intestine consists of the caecum, colon and rectum. The caecum of the rabbit is proportionally the largest of all mammals, with twice the length of the abdominal cavity and 40-60% volume of the gastro-intestinal tract. The normal caecal pH is between 5.9 and 6.8.

The GIT Physiology of the Rabbit (3)

- Due to this complex system, rabbits are prone to frequent GIT pathology conditions that can interfere with the normal functioning of the animal and sometimes lead to mortality
- Gastric stasis and hairballs in the stomach can be the result of low fibre diets, leading to slower emptying of the stomach and caecum. Gastric ulceration may be caused by gastric acid and pepsin that are present in higher concentrations in the rabbit than in other animal species
- GIT hypomotility and stasis of content may lead to caecal impaction and tympany with extensive accumulation of liquid and gas, that in turn leads to dysbiosis of the normal caecal flora, ranging from mild changes to pathogenic bacterial overgrowth with more significant enteritis and life-threatening enterotoxemia
- Typical observations are soft stools, diarrhea or reduced faecal output and decreased body weights and food consumption leading to the deterioration of the general condition of the animal followed by death.

The GIT Physiology of the Rabbit and Chemical Exposure

- Osmotically active and poorly absorbed substances have been demonstrated to cause marked GIT disturbance in rabbits associated with maternal mortality and abortions
- Sucralose orally dosed in pregnant rabbits was shown to be tolerated only at lower doses (≤350 mg/kg bw/day). Higher dosages resulted in maternal mortality and/or abortions that were related to disturbance of the digestion process and not to effects of systemic toxicity
- Other chemical substances that caused mortality in the rabbit through disturbance of the functioning of the GIT:
  - Terbutylazine
  - Spiroxamine
  - Spirodiclofen
  - Certain HIV drugs
- In conclusion, the rabbit has a complex physiology of the GIT which may easily lead to disturbances of the cyclic digestive process under experimental conditions due to intolerance to the test item. Combination with stress conditions such as pregnancy, manipulation and low fibre diets worsen this condition.