Assessment of the toxicological properties of glyphosate by the Pesticides Peer Review

ECHA RAC-39 meeting
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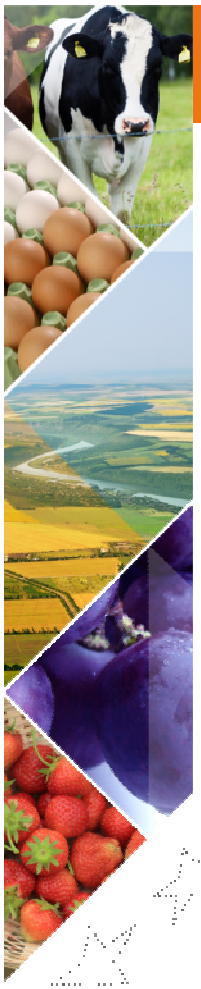

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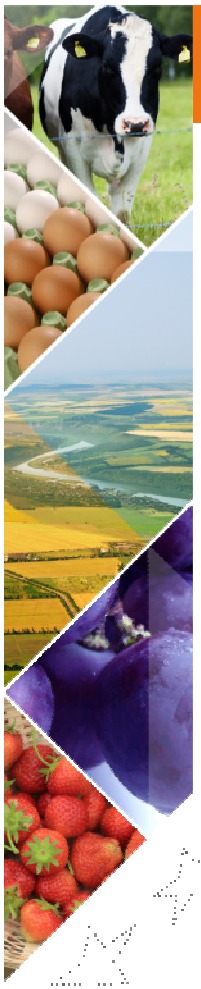


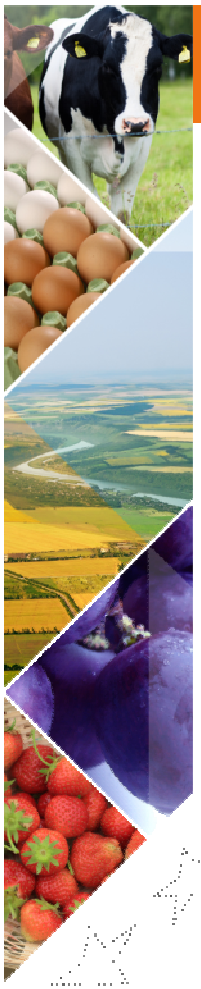
PESTICIDES PEER REVIEW

Proposal for classification HH

Classification and proposed labelling with regard to toxicological data (Annex IIA, point 10)

Substance	glyphosate (acid)
Harmonised classification – Annex VI of Regulation (EC) No 1272/2008[14]	Danger GHS05 (corrosion) Eye Damage 1 H318 - Causes serious eye damage
RMS/peer review proposal[15]	the same as above

- STOT RE 2, H373, proposed in the CLH Report (DE) not discussed during the peer review



OVERVIEW OF THE TOXICOKINETICS

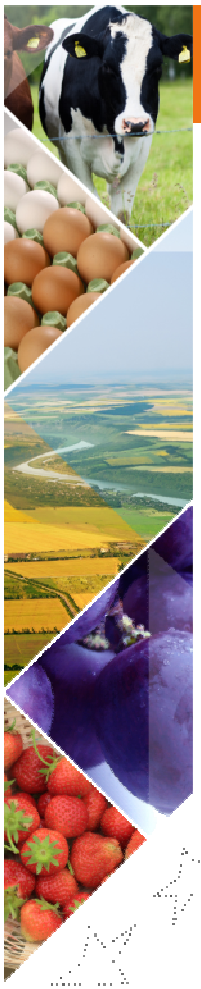
Widely distributed; certain affinity for bones

Mostly eliminated unchanged via faeces with the absorbed dose (20%) recovered in urine

No evidence of accumulation

Rapidly but poorly absorbed (20%)

poorly metabolised (1% AMPA in faeces)



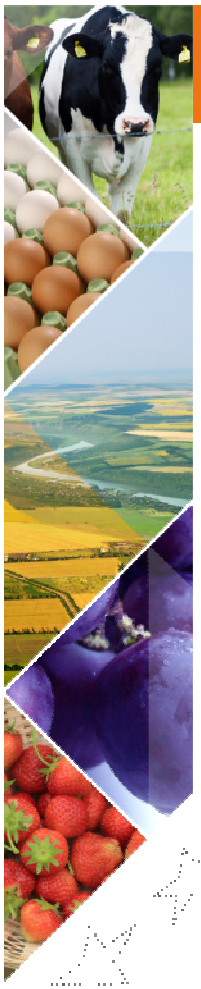
OVERVIEW OF TOXICODYNAMICS

- Low acute toxicity (oral, dermal, inhalation)
- Severely irritant to eyes/mucosa when in the acid form (Eye damage 1 - H318)
- Target organs: **intestinal tract**, salivary glands, liver and urinary bladder; cataracts were observed upon long term exposure

  → Overall short term NOAEL: 300/400/500 mg/kg bw per day in dog/rat/mice

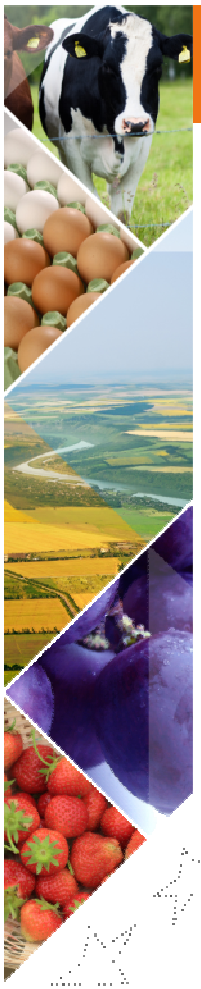
  → Overall long term NOAEL: 100/150 mg/kg bw per day in rat/mice
- Reproductive/offspring effects at high doses
- Developmental toxicity in rabbits at maternally toxic doses (post-implantation loss, ↘ foetal wt & ossification)

  → NOAEL 50 mg/kg bw per day



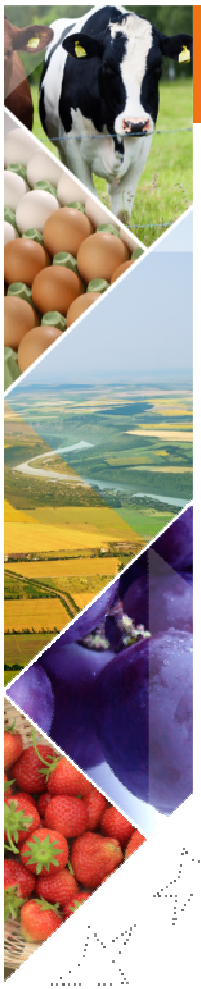
GENOTOXICITY

- Studies conducted with formulations were excluded from this analysis to avoid bias derived from the toxicity of co-formulants.
- Well defined test material is essential to avoid bias from potentially genotoxic impurities (purity and stability).
- Higher representativeness of mammalian systems
- Study design, such as:
  - use of concurrent negative and positive controls in each assay
  - Pre-test determination of cytotoxicity/toxicity to target cell
  - At least 3 analyzable concentrations/dose levels



GENOTOXICITY: *IN VITRO* STUDIES

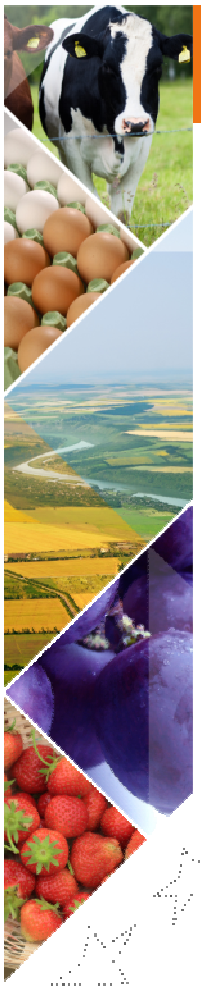
- **Gene mutation**
  - Bacterial assays (Ames tests) and gene mutation in mammalian cells gave consistently negative results
- **Chromosome aberrations**
  - In vitro mammalian chromosome aberration tests performed according to internationally agreed guidelines showed negative results up to 1250 µg/ml.
  - In contrast, 2 non-guideline studies at concentrations of 3-30 and 5-100 µg/ml respectively gave positive results
- **Indicator tests**
  - Mixed outcomes were seen in DNA damage endpoints such as UDS, sister chromatid assay, induction of DNA strand breaks (*in vitro* and *in vivo*) that are considered to give little weight to the overall genotoxicity assessment



GENOTOXICITY: *IN VIVO* STUDIES

chromosome aberration/germ cells

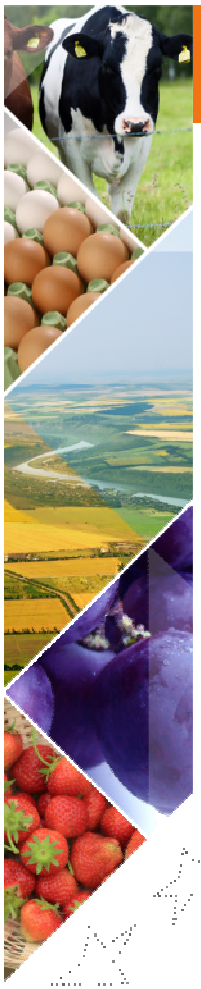
- 7/8 fully acceptable MN/chromosome aberration studies in rats and mice treated by gavage at dose levels up to 2x5000 mg/kg bw gave consistently negative results
- 6 further studies were conducted by the i.p. route, at dose levels exceeding the MTD (up to 1000 mg/kg bw in rats, up to 600 in mice), even so, negative results were obtained, except in 2 studies with methodological deficiencies.
- 2 negative germ cells mutagenicity



GENOTOXICITY: WEIGHT OF EVIDENCE

- 1 weak positive response in 8 studies (p.o.) observed at the high dose (2x5000 mg/kg bw) in ♀ only, with high SD, not reproduced in ♂.
- 2/6 i.p. studies positive at doses exceeding the ip LD_{50} in studies presenting methodological drawbacks:
  - No reference to TG, not GLP, reporting deficiencies in both studies
  - Second study with major drawbacks including scoring of total erythrocytes instead of immature PCE for micronuclei
- DNA damage observed at high or toxic doses due to cytotoxicity rather than DNA interaction.

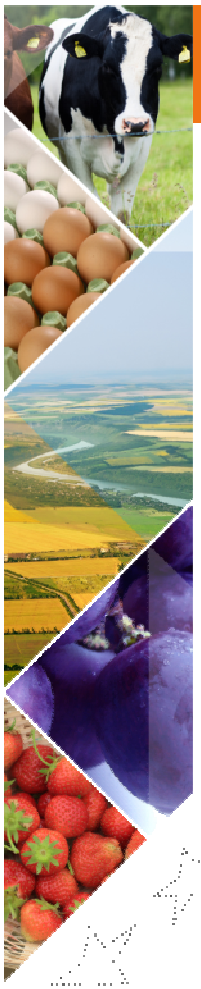
Glyphosate is unlikely to be genotoxic



ANIMAL DATA ON CARCINOGENICITY

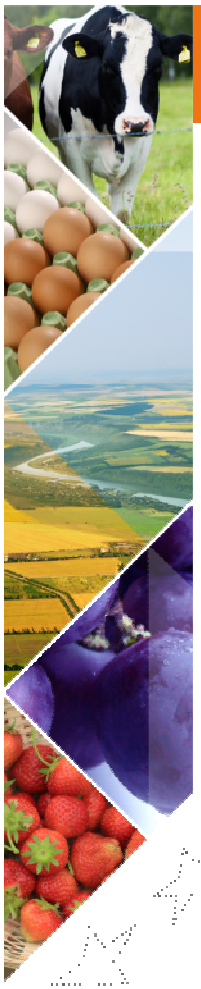
Overview of long term rat studies available to the peer review

- 12 studies in rats
  - 6 acceptable studies (3 in Wistar rats and 3 in SD rats (Stout & Ruecker, 1990, Atkinson, 1993, Suresh, 1996, Enomoto, 1997, Brammer, 2001, Wood, 2009)
  - 2 supplementary studies (Lankas, 1981, Milburn, 1996)
  - 4 studies are inadequate (Calandra, 1974, Bhide, 1997, Chruscielska et al 2000, Seralini, 2012)



WEIGHT OF EVIDENCE ON THE TUMOUR INCIDENCE IN RATS

- Increased tumour incidences in rats were not considered toxicologically relevant as:
  - Limited to a supplementary study and the older study in 6 acceptable studies
  - No dose-response in a statistically significant increase (pair-wise comparison) of the incidence of pancreatic islet cell adenomas in males (2 studies, one of which supplementary)
  - Statistically significant increased incidence of testicular interstitial cell tumours not reproduced in 6 long term studies using much higher dose levels.
  - Statistically significant linear trend for hepatocellular adenomas in males and thyroid C-cell adenomas in females corresponding to marginal trends in benign tumours limited to one sex, not reproduced among 5 long term studies; not confirmed by a statistical analysis in a pair-wise comparison
  - No pre-neoplastic lesion or progression to malignancy



ANIMAL DATA ON CARCINOGENICITY

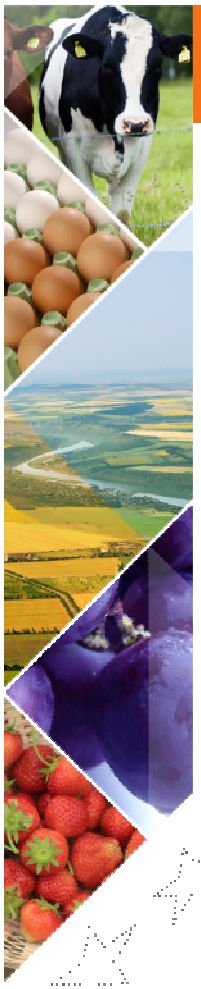
Overview of long term mice studies available to the peer review

- 8 studies in mice
  - 4 acceptable studies (in CD-1 mice) (Knezevich & Hogan, 1983; Atkinson, 1993; Sugimoto, 1997; Wood, 2009)
  - 1 study of doubted reliability after consideration by the peer review (Kumar, 2001)
  - 3 studies are inadequate (Vereczkey and Csanyi, 1982; Bhide, 1988; George, 2010)

REVIEW OF MALIGNANT LYMPHOMAS IN MICE

Study	Dose levels mg/kg bw per d	NOAEL/ LOAEL	Males	Females
Knezevich & Hogan, 1983	CD-1 0, 157, 814, **4841**	157/ 814	2/48 – 5/49 – 4/50 – 2/49 (4%) (10%) (8%) (4%)	6/50 – 6/48 – 7/49 – 11/49 (12%) (12%) (14%) (22%)
Atkinson, 1993	CD-1 0, 100, 300, **1000**	1000/ >1000	4/50 – 2/50 – 1/50 – 6/50 (8%) (4%) (2%) (12%)	14/50 – 12/50 – 9/50 – 13/50 (28%) (24%) (18%) (26%)
Sugimoto, 1997	CD-1 (ICR) 0, 153, 787, **4348/4116**	153/ 787	2/50 – 2/50 – 0/50 – 6/50 * (4%) (4%) (12%) [HCD: 4-19% - mean 6.3%]	6/50 – 4/50 – 8/50 – 7/50 (12%) (8%) (16%) (14%) [HCD: 8-27% - mean 15%]
Wood, 2009	CD-1 (ICR) 0, 71, 234, **810**	810/ >810	0/51 – 1/51 – 2/51 – 5/51 * (2%) (4%) (10%) [no valid HCD]	11/51 – 8/51 – 10/51 – 11/51 (22%) (16%) (20%) (22%)
Kumar, 2001	Swiss albino 0, 15, 151, **1460**	151/ 1460	10/50 -15/50 - 16/50 - 19/50 ** (20%) (30%) (32%) (38%) [HCD: 6-30% - mean 18.4]	18/50 - 20/50 - 19/50 - 25/50** (36%) (40%) (38%) (50%) [HCD: 14-58% - mean 41.6%]

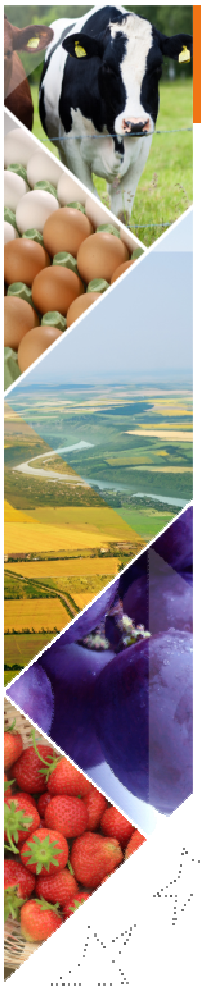
* statistically significant according to Cochran-Armitage test for linear trend
** statistically significant in Z-test although not in Fisher's exact test or linear trend



REVIEW OF MALIGNANT LYMPHOMAS IN MICE

Weight of evidence/expert judgment

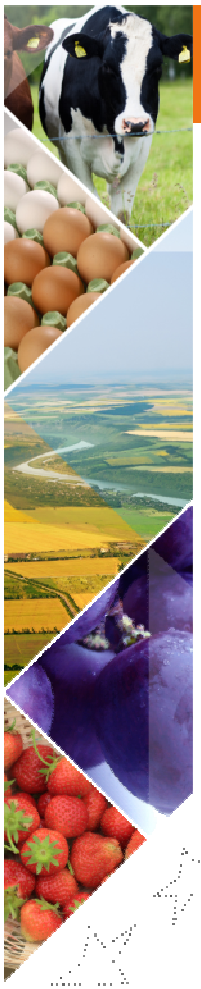
- Malignant lymphomas are one of the most common neoplasms in CD-1 mice, females being more prone to this tumour type than males
- The one instance of statistical significance according to pair-wise comparison (and outside of HCD) was recorded at high dose level in a study probably affected by murine oncogenic virus
- Inconsistency in results among 5 studies in particular when comparing similar dose levels
- The finding is not affecting animal survival and there was no change in tumour latency
- Overall incidences are within HCD even at the highest dose tested, although one study lack of valid HCD
- Minority view in the peer review considered that this finding may require classification as a Carc. Cat. 2



OTHER TUMOURS IN MICE

Renal tubular tumours in males

- Statistically significant linear trends in males were considered not toxicologically relevant as:
  - observed only at high dose (>4000 mg/kg bw per day), above the MTD and same incidence as controls in other studies
  - No statistical significance in pair-wise comparison to controls when adjusted for other variables (such as higher survival in the high dose group)
  - Adenomas were not associated with pre-neoplastic changes (i.e. tubular cell hyperplasia) as it would be expected if treatment related

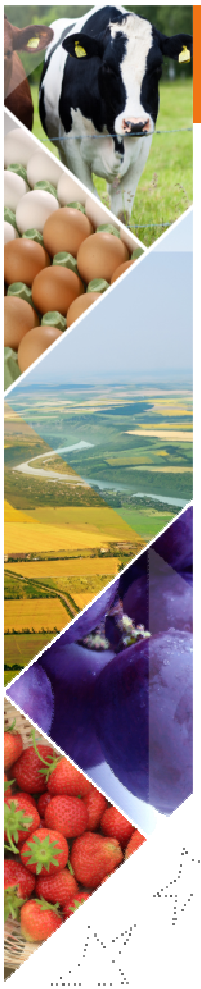


OTHER TUMOURS IN MICE

Haemangiosarcomas in males

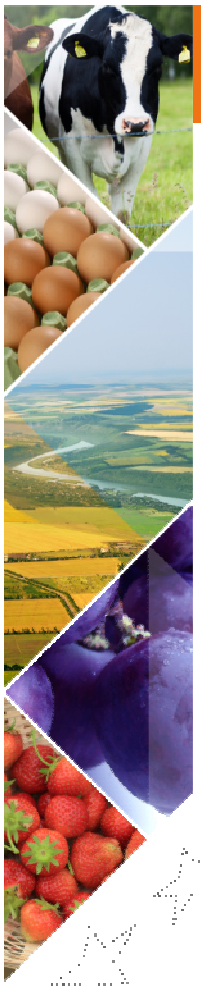
- Statistically significant linear trends of haemangiosarcomas were not considered toxicologically relevant as:
  - Incidences observed at the highest dose were within the range of HCD in one study
  - In the other study although no valid HCD was available, lower incidences were observed at high dose (>4000 mg/kg bw per day), above the MTD
  - No statistical significance in a pair-wise comparison
  - Although circumstantial, no blood and/or endothelial toxicity was observed with glyphosate

Considering animal data on carcinogenity, glyphosate is unlikely to pose a carcinogenic hazard



EPIDEMIOLOGICAL STUDIES

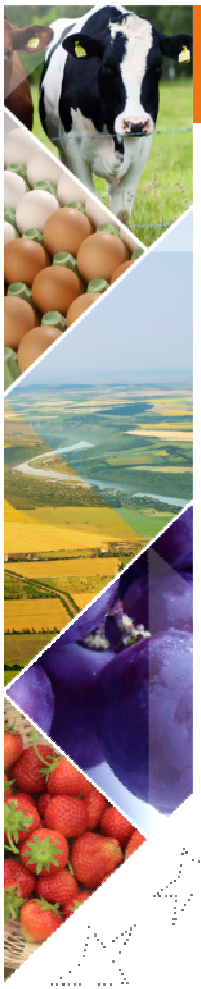
- Cohort studies (10 studies based on AHS)
  - ➢ Glyphosate did not cause/increase the risk of all cancers
    - Interpretation of multiple myeloma is limited
- Case-control studies
  - 14 studies on lymphoid neoplasms
    - Non-Hodgkin lymphoma
    - Multiple myeloma
    - leukaemia
  - 5 on other cancer sites
  - Meta-analysis
  - ➢ Slight, non-statistically significant ↗ OR for an association between glyphosate exposure and NHL were observed in few cases



EPIDEMIOLOGICAL STUDIES

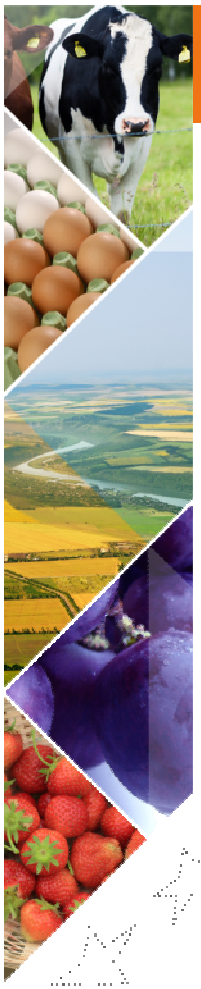
- Weight of evidence
  - The lack of consistency in the results (few cases, limited increases in ORs and/or ORs not statistically significant
  - Lack of positive association in the Cohort study
  - Limitations inherent to epidemiological studies
    - Confounders, including co-formulants, multiple exposure, other risk factors
    - Exposure difficult to measure, use of interview/questionnaires subject to recall bias, no measures from biomarkers
    - Classification of cancers changing over time and/or not reported from official records

EPIDEMIOLOGICAL STUDIES



Conclusion

- there is **very limited evidence** for an association between glyphosate-based formulations and NHL
- Overall evidence is **inconclusive for a causal link** or otherwise convincing associative relationship between glyphosate and cancer in human studies



DEVELOPMENTAL TOXICITY IN RABBITS

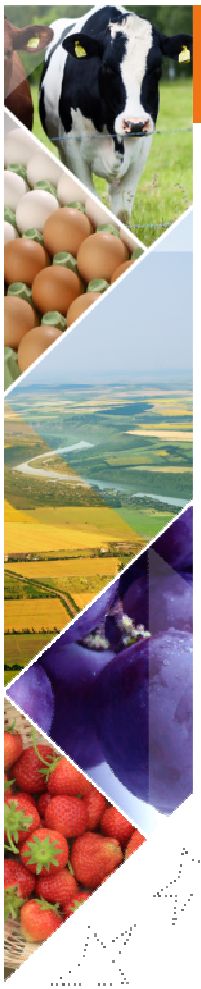
Overview of developmental toxicity studies in rabbits

- 4 studies acceptable (Brooker 1991; Hojo, 1995; Coles and Doleman 1996; Moxon, 1996)
- 3 studies supplementary (Tasker 1980; Bhide & Patil, 1989; Suresh 1993)
- 1 study inappropriate (Anonym, 1981)

Pregnant rabbits are particularly vulnerable to glyphosate administration

➡ excessive toxicity (mortality) observed in 5/7 studies

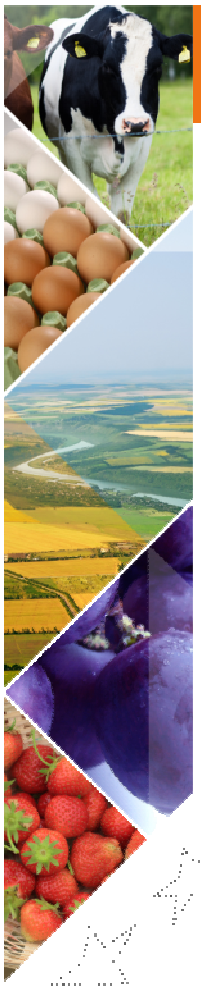
Associated with no dev effects (2 studies), reduced foetal weight and retarded ossification (1 study) and post implantation losses (1 study)



DEVELOPMENTAL TOXICITY IN RABBITS

Developmental effects - heart

- ↗ incidence cardiac malformation (mainly interventricular septal defect), late embryonic death and post-implantation losses at high dose level (Brooker 1991)
- ↗ ventricular septal defects at high dose, other external, visceral & skeletal malformations, death (suppl. Bhide & Patil, 1989)
- ↗ incidence dilated heart was increased in the high dose group despite a low number of foetuses and litters and maternal mortality (>50%) (suppl. Suresh 1993)



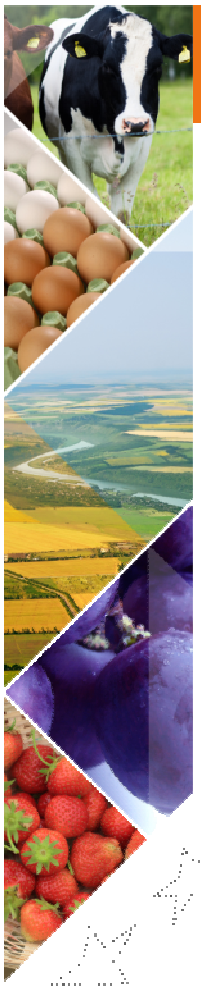
DEVELOPMENTAL TOXICITY IN RABBITS

Heart effects - WoE experts judgment

- Effects were consistently observed at doses causing excessive maternal toxicity (death)
- Effects were observed in the 3 older studies, not reproduced in the 3 most recent studies
- 2 instances of cardiac effects reported in supplementary studies, 1 with serious reporting deficiencies and 1 with small number of litters for examination (low pregnancy rate, lethality and reporting deficiencies)

→ No classification regarding developmental toxicity is proposed by the majority of peer review experts

Minority view considered that glyphosate may require classification regarding developmental toxicity



HAZARD CHARACTERISATION OF GLYPHOSATE

Glyphosate is unlikely to be genotoxic, neurotoxic or toxic for the reproduction or development and is unlikely to pose a carcinogenic hazard to humans

ADI	• 0.5 mg/kg bw per day • Developmental toxicity, rabbit • Uncertainty factor 100
ARfD	• 0.5 mg/kg bw • Developmental toxicity, rabbit • Uncertainty factor 100
AOEL	• 0.1 mg/kg bw per day • Developmental toxicity, rabbit • Uncertainty factor 100/20% OA

- EFSA recommends that the toxicity of each formulation and particularly genotoxic potential be further considered and addressed by MS

Thank you

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