EU assessment of the carcinogenic potential of glyphosate

FIFRA Scientific Advisory Panel on Carcinogenic Potential of Glyphosate, 13-16 December 2016

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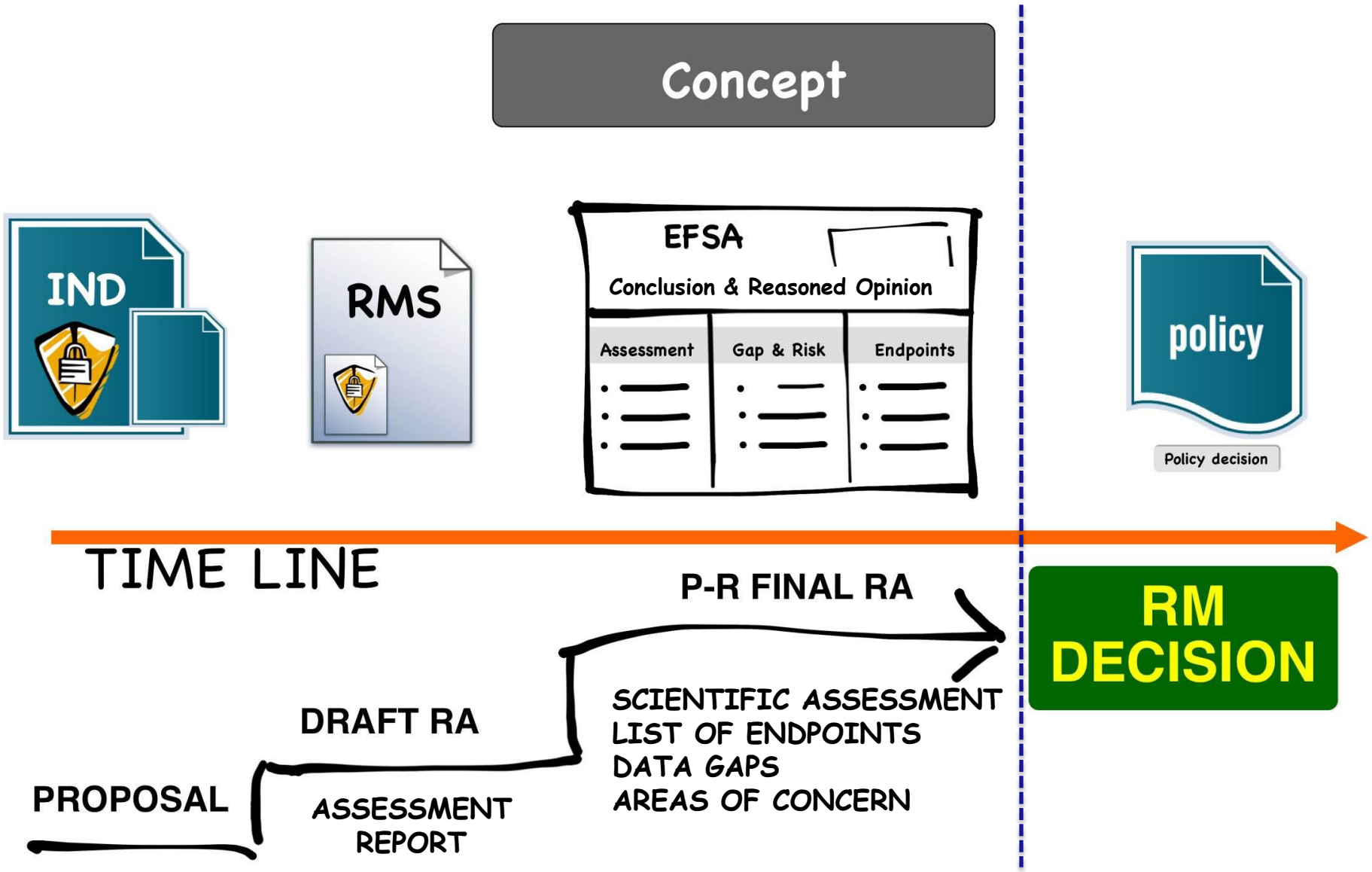
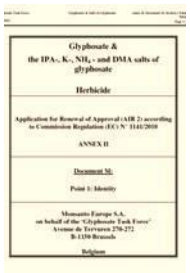
PESTICIDES PEER-REVIEW in the EU



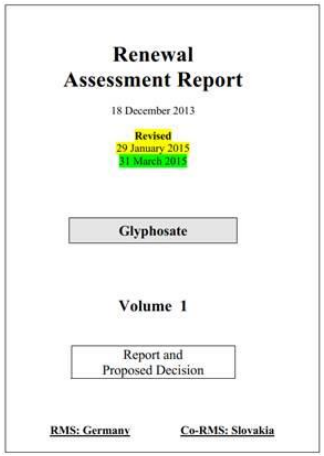
EU PEER REVIEW - GLYPHOSATE

- 2012–2013: First assessment by the Rapporteur Member State (RMS: Germany). Renewal assessment report (RAR) sent to EFSA
- 2014: Peer review with all Member States begins; public consultation launched on RAR
- 2015:
  - **First revision of the RAR**
  - Feb/March: **Expert consultations** with Member States on mammalian toxicology, residues, ecotoxicology, environmental fate
  - **Second revision of the RAR**
  - April: EFSA receives mandate from the Commission to review IARC conclusion on carcinogenicity; work begins in August when IARC Monograph published
  - **Addendum 1 to the RAR**
  - Aug/Sept: EFSA runs further **expert consultations** on carcinogenicity
  - October: final consultation with Member States; adoption of EFSA Conclusion

PEER REVIEW DOCUMENTS



RMS → Comments → EFSA



Mandatory GLP studies* published scientific literature** other evaluations

RMS evaluation, updates are highlighted

Comments, responses, meeting reports, MSs views

Critical concerns, data gaps. Validated endpoints

* Commission Regulations (EU) No. 544/2011 and 545/2011 of 10 June 2011

**EFSA Guidance on submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (EFSA Journal 2011;9(2):2092)

GLYPHOSATE DOSSIER

- >700 studies and references considered in the RAR (revised in January and March 2015) in the mammalian toxicology section
  - 20 long term/carcinogenicity studies
  - 107 genotoxicity studies
  - 30 epidemiological studies
- 11 additional studies were considered in the addendum assessing the IARC conclusion(August 2015)
  - 3 Reanalysis of the AHS prospective cohort
  - 6 case-control studies
  - 2 publications on genotoxicity

OVERVIEW OF THE TOXICOKINETICS

Rapidly but poorly absorbed (20%)

poorly metabolised (1% AMPA in faeces)

Widely distributed; certain affinity for bones

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

No evidence of accumulation

OVERVIEW OF TOXICODYNAMICS

- Low acute toxicity (oral, dermal, inhalation)
- Severely irritant to eyes/mucosa when in the acid form
- 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

- *In vitro* studies
  - Gene mutation in bacterial and mammalian cells
  - Chromosome aberration
  - Indicative tests
- *In vivo* studies
  - Indicative tests
  - In somatic cells (micronucleus/chromosome aberration)
  - In germ cells
- Weight of evidence

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

IN VITRO STUDIES

Gene mutation

- Bacterial assays (Ames tests) gave consistently negative results
  - 15 fully acceptable studies and 3 supplementary studies are reported in DAR/RAR
- Gene mutation tests in mammalian cells gave consistently negative results
  - 5 fully acceptable studies and 1 supplementary study reported in DAR/RAR

IN VITRO STUDIES

Chromosome aberration

- *In vitro* mammalian chromosome aberration tests performed according to internationally agreed guidelines showed negative results up to 1250 µg/ml.
  - 3 fully acceptable studies and 1 supplementary.
- In contrast, 2 non-guideline studies at concentrations of 3-30 and 5-100 µg/ml respectively gave positive results

IN VITRO/IN VIVO STUDIES

Indicator tests

- Negative *in vitro* UDS (1 guideline and 1 non-guideline study)
- Positive SCE tests (2 non-guideline studies)
- Positive results for induction of DNA strand breaks *in vitro* (5 non-guideline studies)
- Induction of DNA strand breaks was reported in 2 publications following *in vivo* high i.p. dosing (above i.p. LD_{50}) or repeated oral dosing (methodological deficiencies)

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

Carcinogenicity assessment

- Assessment of the quality of the study
  - Design, conduct and reporting of the study
  - Well defined test material
- Interpretation of the study results
  - Dose-response curve
  - Weight of the trend analysis vs. pair-wise comparison for adjustment to other variables
  - Appropriate historical control data from the same strain, same performing laboratory and contemporaneous to the study (around 5 years)
  - Considerations of a plausible mode of action
  - Reduced latency/progression to malignancy
  - Concomitant toxicity (MTD)

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)

REVIEW OF RAT TUMOUR INCIDENCE

Study	Dose levels mg/kg bw per d	NOAEL/ LOAEL	Tumour	Incidence
Lankas, 1981 (1)	0, 3, 10.3, **31.5**	31.5/ >31.5	Pancreatic islet cell adenomas	Males: 0/50 – 5/49* – 2/50 – 2/50 (10%) (4%) (4%)
			Testicular interstitial cell tumours	Males: 0/50 – 3/50 – 1/50 – 6/50* (6%) (2%) (12%)
Stout & Ruecker, 1990 (2)	0, 89/113, 362/457, **940/1183** (m/f)	89/ 362	Pancreatic islet cell adenomas	Males: 1/43 – 8/45* – 5/49 – 7/48* (2%) (18%) (10%) (15%)
			Hepatocellular adenomas	Males: 2/44 – 2/45 – 3/49 – 7/48 ** (5%) (4%) (6%) (15%)
			Thyroid C-cell adenomas	Females: 2/60 – 2/60 – 6/60 – 6/60 ** (3%) (3%) (10%) (10%)

(1) Supplementary study, not according to current standards
(2) Survival was very low (<50%) in all groups: 44 – 44 – 34 – 36%
* statistically significant according to Fischer's exact test
** statistically significant according to Cochran-Armitage test for linear trend

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

REVIEW OF RENAL TUBULAR TUMOURS IN MICE

Study	Dose levels mg/kg bw per d	NOAEL/ LOAEL	Males	Females
Knezevich & Hogan, 1983 (1)	CD-1 0, 157, 814, **4841**	157/ 814	1/49 – 0/49 – 1/50 – 3/50 * (adenomas + carcinomas combined at re-examination)	0/50 – 0/50 – 0/50 – 0/50
Atkinson, 1993	CD-1 0, 100, 300, **1000**	1000/ >1000	2/50 – 2/50 – 0/50 – 0/50 (1 adenoma + 1 carcinoma at each control and low-dose)	0/50 – 0/50 – 0/50 – 0/50
Sugimoto, 1997	CD-1 (ICR) 0, 153, 787, **4348/4116**	153/ 787	0/50 – 0/50 – 0/50 – 2/50 * (adenomas) (4%)	0/50 – 0/49 – 0/50 – 0/50
Wood, 2009	CD-1 (ICR) 0, 71, 234, **810**	810/ >810	0/51 – 0/51 – 0/51 – 0/51	0/51 – 0/51 – 0/51 – 0/51
Kumar, 2001	Swiss albino 0, 15, 151, **1460**	151/ 1460	0/50 – 0/50 – 1/50 – 2/50 * (adenomas) (2%) (4%)	0/50 – 0/18 – 0/21 – 0/50

(1) Re-evaluated by PWG

* statistically significant according to Cochran-Armitage test for linear trend

REVIEW OF RENAL TUMOUR INCIDENCE IN MICE

Weight of evidence/expert judgment

- 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 - Knezevich & Hogan)
  - Adenomas were not associated with pre-neoplastic changes (i.e. tubular cell hyperplasia) as it would be expected if treatment related

REVIEW OF HAEMANGIOSARCOMAS IN MICE

Study	Dose levels mg/kg bw per d	NOAEL/ LOAEL	Males	Females
Knezevich & Hogan, 1983 [A]	CD-1 0, 157, 814, **4841**	157/ 814	0/48 – 0/49 – 1/50 – 0/49 (2%)	1/50 – 0/50 – 2/49 – 1/49 (2%) (4%) (2%)
Atkinson, 1993 [B]	CD-1 0, 100, 300, **1000**	1000/ >1000	0/50 – 0/50 – 0/50 – 4/50 * (8%) [HCD: 0 – 8%]	0/50 – 2/50 – 0/50 – 1/50 (4%) (2%) [HCD: 0 – 4%]
Sugimoto, 1997[B]	CD-1 (ICR) 0, 153, 787, **4348/4116**	153/ 787	0/50 – 0/50 – 0/50 – 2/50 * (4%)	0/50 – 0/50 – 0/50 – 0/50
Wood, 2009 [C]	CD-1 (ICR) 0, 71, 234, **810**	810/ >810	2/51 – 1/51 – 2/51 – 1/51	0/51 – 1/51 – 0/51 – 0/51 (2%)
Kumar, 2001	Swiss albino 0, 15, 151, **1460**	151/ 1460	0/29 – 0/29 – 1/27 – 0/23	1/35 – 0/32 – 0/28 – 0/30 (3%)

* statistically significant according to Cochran-Armitage test for linear trend

A in spleen

B in vascular system

C in liver and/or kidney

REVIEW OF HAEMANGIOSARCOMAS

Weight of evidence/expert judgment

- 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, incidences were lower than the ones 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 Non-Hodgkin Lymphoma
- Overall evidence is **inconclusive for a causal link** or otherwise convincing associative relationship between glyphosate and cancer in human studies.

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

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

EU status

- Standing Committee on Plants, Animals, Food and Feed, Section Phytopharmaceuticals - Plant Protection Products – Legislation

  - in June 2016 postponed its decision regarding glyphosate's renewal of approval (extended the current approval period until 31/12/2017)
  - awaiting the conclusion of the Risk Assessment Committee at the European Chemicals Agency who is responsible to harmonise classification and labelling of chemicals in the EU according to Regulation (EC) 1272/2008 (CLP Regulation)

Thank you

European Food Safety Authority

www.efsa.europa.eu

SUPPLEMENTARY INFORMATION

Overview of available animal carcinogenicity studies

OVERVIEW OF CARCINOGENICITY STUDIES IN RATS

Study	Study type	Dose levels mg/kg bw per d	NOAEL/ LOAEL	Toxicity / MTD	Tumour effect
Calandra, 1974	Study not acceptable: Deficient study, not guideline compliant, dose levels much too low for meaningful evaluation				
Bhide, 1997	Study not acceptable: Study design/reporting inadequate, including lack of information on test material, low number of animal undergoing histopathology				
Chruscielska *et al* 2000	Study not acc. Apparent use of a glyphosate formulation, unknown actual dose level to which the animals were exposed to, limited details available in the publication on the study design				
Seralini, 2012	Study not acceptable: Study design/reporting inadequate for the evaluation of glyphosate carcinogenicity, use of glyphosate formulation				
Lankas, 1981 (1)	26mo, SD rat	0, 3, 10.3, 31.5	31.5/ >31.5	No adverse effects No MTD	No effect *
Milburn, 1996 (2)	1yr, Wistar rat	0, 141, 560, 1409	141/ 560	Toxicity study high dose > MTD	No effect

(1) Supplementary study, dose levels tested were too low, far below an MTD; study flawed by serious reporting deficiencies

(2) Supplementary study due to shorter duration than required for assessment of carcinogenicity

* See detailed assessment

OVERVIEW OF CARCINOGENICITY STUDIES IN RATS

Study	Study type	Dose levels mg/kg bw per d	NOAEL/ LOAEL	Toxicity / MTD	Tumour effects
Stout & Ruecker, 1990	2yr, SD rat, combined	0, 89, 362, 940	89/ 362	LOAEL: stomach mucosal inflammation High dose > MTD	No treatment-related effect* (3)
Atkinson, 1993	2yr, SD rat, combined	0, 10, 100, 300, 1000	100/ 300	LOAEL: salivary gland findings ↑AP and↑ liver weight High dose > MTD (↓Bw)	No effect
Suresh, 1996	2yr, Wistar rat, combined	0, 6.3, 59.4, 595.2	60/ 595	LOAEL: Cataracts, ↑ AP No MTD	No effect
Enomoto, 1997	2yr, SD rat, combined	0, 104, 354, 1127	104/ 354	LOAEL: ↓Bw/bw gain, ↓ food efficiency, gastro-intestinal effects High dose > MTD	No effect (3)

(3) with a poor survival (<50%) in control and treated animals

* See detailed assessment

OVERVIEW OF CARCINOGENICITY STUDIES IN RATS

Study	Study type	Dose levels mg/kg bw per d	NOAEL/ LOAEL	Toxicity / MTD	Tumour effects
Brammer, 2001	2yr, Wistar rat, combined	0, 121, 361, 1214	361/ 1214	LOAEL: ↓Bw, food efficiency, clinical chemistry and histopathology findings regarding the liver, kidneys High dose > MTD	No effect
Wood, 2009	2yr, Wistar rat, combined	0, 86, 285, 1077	285/ 1077	LOAEL: Bw gain↓, ↑AP , kidney and skin effects MTD reached	No effect

- Overall, a robust assessment on glyphosate carcinogenicity was performed on 6 valid studies in rats, no toxicologically relevant increase in tumour incidences was observed

OVERVIEW OF CARCINOGENICITY STUDIES IN MICE

Studies of doubted reliability or found unacceptable (in red):

Study	Study type	Dose levels mg/kg bw per d	NOAEL/ LOAEL	critical effect at the LOAEL
Kumar, 2001*	**18 mo**, Swiss albino, carcino	0, 15, 151, 1460	151/1460	↑ incidence of malignant lymphoma** outside HCD for males; ↑ cystic glands in stomach
Vereczkey and Csanyi, 1982	Study design and reporting with serious deficiencies Such as: only 2 dose levels included (100 and 300 ppm), too low number of surviving animals examined for pathological examination.			
Bhide, 1988	Study design and reporting with serious deficiencies Such as: low number of animals, dose levels too low (75, 150 and 300 ppm – actual intake not calculated), limited number of haematological and biochemistry investigations, some organs not examined pathologically			
George, 2010	Study conducted with formulation to evaluate tumour promotion, inadequate for the evaluation of glyphosate carcinogenicity			

* Study found unreliable after detailed assessment, due to the occurrence of viral infection in all groups including controls

** statistically significant (Z-test pair-wise comparison although not in Fisher's exact test or linear trend)

OVERVIEW OF CARCINOGENICITY STUDIES IN MICE

Acceptable studies :

Study	Study type	Dose levels mg/kg bw per d	NOAEL/ LOAEL	critical effect at the LOAEL
Knezevich & Hogan, 1983	**2 yr**, CD-1 carcino/ chronic	0, 157, 814, 4841	157/814	Males: ↓ bw, hepatocellular centrilobular hypertrophy and bladder epithelial hyperplasia MTD reached
Atkinson, 1993	**2 yr**, CD-1, carcino	0, 100, 300, 1000	1000/>1000	Equivocal thymus findings, not associated with histopathological findings (common in mice), no MTD
Sugimoto, 1997	**18 mo**, CD-1 (ICR), carcino	0, 153, 787, 4116	153/787	Bw gain, ↓ food cons & effic, gastro-intestinal effects High dose > MTD
Wood, 2009	**18 mo**, CD-1 (ICR), carcino	0, 71, 234, 810	810/>810	No effect observed, no MTD

- Overall, a robust assessment on glyphosate carcinogenicity was performed on 4 valid studies in mice, no toxicologically relevant increase in tumour incidences was observed