Glyphosate AIR 2022 Pre-submission Meeting

18 October 2019

[redacted], PhD, DABT
Glyphosate Taskforce 2
Toxicology Technical Working Group
(GTF2 ToxTWG)

Toxicology Technical Working Group (Tox TWG)

- BAYER (chair): [redacted]
- SYNGENTA: [redacted]
- ALBAUGH: [redacted]
- NUFARM: [redacted]
- ARYSTA: [redacted]
- KNOELL: [redacted]

Perspect Med Educ (2016) 5:320–322
DOI 10.1007/s40037-016-0307-z

COMMENTARY

What is science without replication?

Jimmie Leppink[1] · Patricia Pérez-Fuster[2]

Published online: 26 October 2016

The Latin adagio *unus testis nullus testis* is an important principle that equally applies to criminal law as to science: we should not base our conclusions on a single piece of evidence [1]. Of course, criminal law and science are very different practices. After all, while a criminal case typically revolves around the evaluation of evidence in favour of and against competing hypotheses about what occurred in a particular case with specific actors, the goal of science is to establish generally applicable laws and principles. Yet, what unites the two practices is that both are about establishing a *chain of evidence*: pieces of evidence have to be anchored as narratives into a story line that increases the plausibility of some hypothesis relative to competing hypotheses. Just like a DNA match from a cigarette found at a crime scene cannot be sufficient to conclude on the guilt of the suspect in the absence of contextual information on how the cigarette got there (e.g. eyewitness testimonies), the meaning of findings from a scientific study cannot be established without considering context, theory and relevant previous research. In other words, science too is about storytelling [2].

In their article *Science: The slow march of accumulating evidence* [3], Picho, Maggio and Artino discuss a very powerful if not the most powerful tool for establishing chains of evidence and solid stories in a scientific research field: replication. We fully support the authors' plea to treat replication as essential to the accumulation of knowledge in the field of medical education and would like to take it even one step further by arguing that the potential of replication is even bigger than discussed in the vast body of literature on replication in education and psychology thus far.

Why the potential of replication is even bigger than discussed thus far

Picho and colleagues distinguish between direct and conceptual replication. While the former comes down to repeating a study as closely as possible, the latter is about attempts to test the theory that underlies particular findings. The authors provide excellent arguments for why especially conceptual replication can help improve the quality of research in a field. Take for instance the so-called *expertise reversal effect* [4]: instructional support that is beneficial for novice learners loses its effectiveness or even becomes detrimental as learners become more proficient. This effect has been replicated for different types of learners in different domains and therefore has clear implications for educational practice and research in these domains.

However, we would like to argue that both direct and conceptual replication have a use for at least one common reason: different studies are carried out with different participants and therefore always yield somewhat different results. In sampling theory, this is also referred to as *sampling error*. Under the assumption of random sampling, which underlies the frequently reported *p*-values and many other statistics, findings from individual studies and hence differences between studies are to some extent always due to chance. This sample-to-sample fluctuation is especially

Department and institution to which the work should be attributed: School of Health Professions Education, Maastricht University, The Netherlands.

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Perspect Med Educ (2016) 5:320-322

What is science without replication?

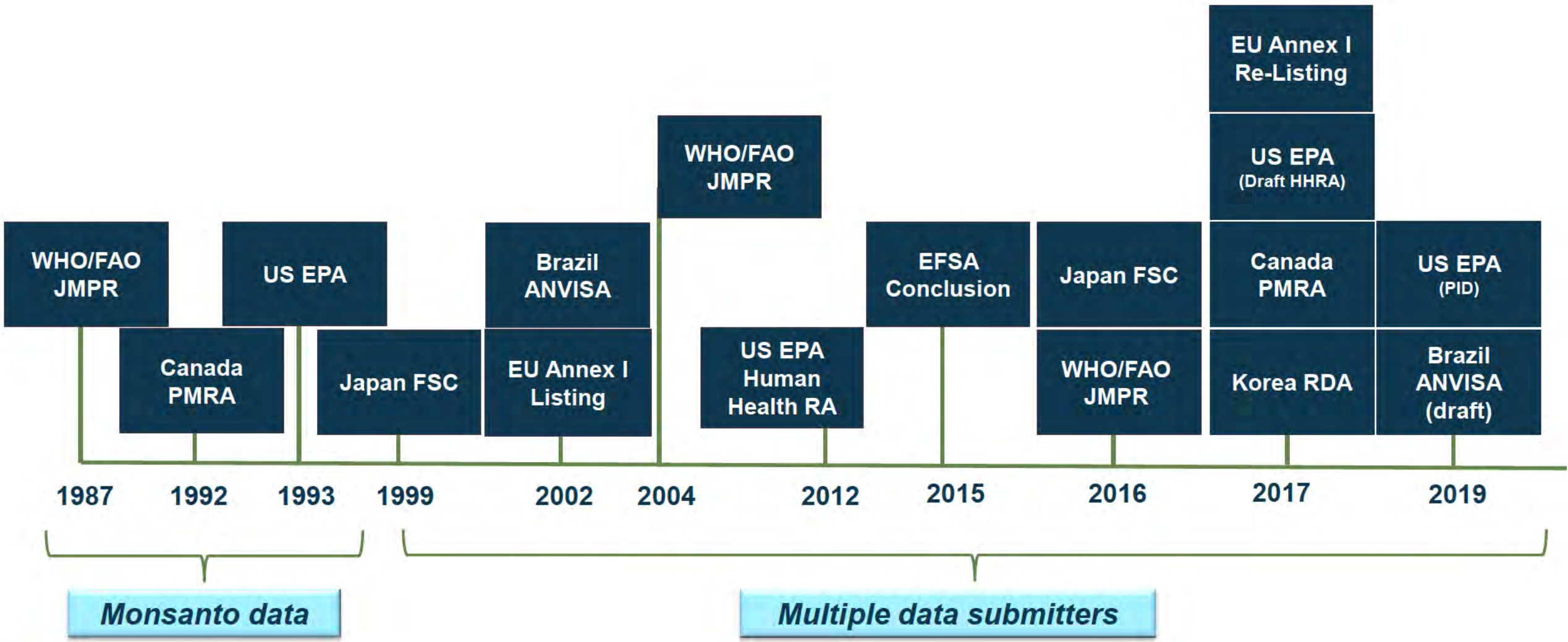
unis testis nullus testis

one witness is not a witness

…the meaning of findings from a scientific study cannot be established without considering context, theory and relevant previous research.

Funding: Netherlands Organisation for Scientific Research

Key Regulatory Reviews of Glyphosate



Recent Regulator Conclusions

efsa
European Food Safety Authority
EFSA Journal 2015;13(11):4302

CONCLUSION ON PESTICIDE PEER REVIEW

Conclusion on the peer review of the pesticide risk assessment of the active substance glyphosate[1]

European Food Safety Authority (EFSA)[2]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

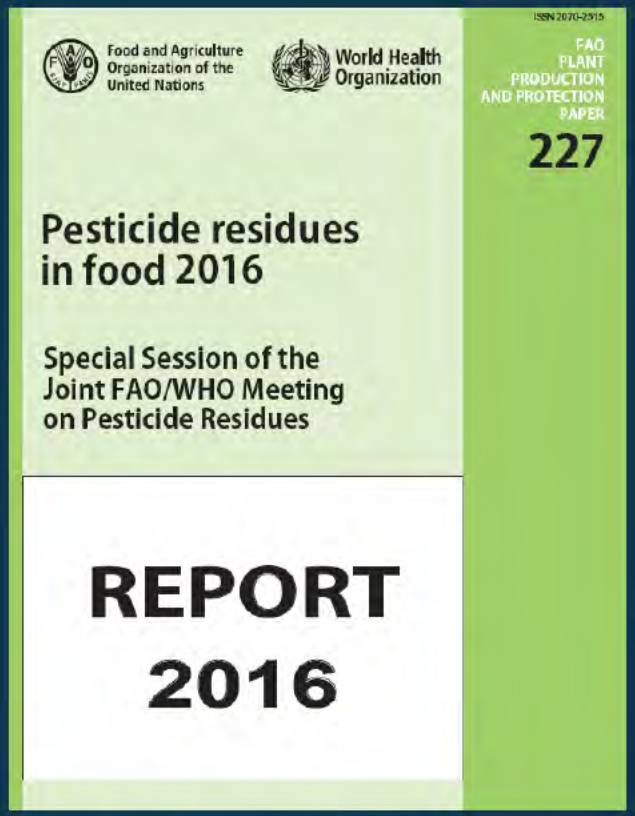
The conclusions of the European Food Safety Authority (EFSA), following the peer review of the initial risk assessments carried out by the competent authority of the rapporteur Member State Germany, for the pesticide active substance glyphosate are reported. The context of the peer review was that required by Commission Regulation (EU) No 1141/2010 as amended by Commission Implementing Regulation (EU) No 380/2013. The conclusions were reached on the basis of the evaluation of the representative uses of glyphosate as a herbicide on emerged annual, perennial and biennial weeds in all crops [crops including but not restricted to root and tuber vegetables, bulb vegetables, stem vegetables, field vegetables (fruiting vegetables, brassica vegetables, leaf vegetables and fresh herbs, legume vegetables), pulses, oil seeds, potatoes, cereals, and sugar- and fodder beet; orchard crops and vine, before planting fruit crops, ornamentals, trees, nursery plants etc.] and foliar spraying for desiccation in cereals and oilseeds (pre-harvest). The reliable endpoints, concluded as being appropriate for use in regulatory risk assessment and derived from the available studies and literature in the dossier peer reviewed, are presented. Missing information identified as being required by the regulatory framework is listed. Concerns are identified. Following a second mandate from the European Commission to consider the findings from the International Agency for Research on Cancer (IARC) regarding the potential carcinogenicity of glyphosate or glyphosate-containing plant protection products in the on-going peer review of the active substance, EFSA concluded that glyphosate is unlikely to pose a carcinogenic hazard to humans and the evidence does not support classification with regard to its carcinogenic potential according to Regulation (EC) No 1272/2008.

© European Food Safety Authority, 2015

KEY WORDS

glyphosate, peer review, risk assessment, pesticide, herbicide

EFSA concluded that glyphosate is ***unlikely to pose a carcinogenic hazard to humans and the evidence does not support classification*** *with regard to its carcinogenic potential according to Regulation (EC) No 1272/2008.* ***EFSA – Approved October 2015***



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World Health Organization

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227

Pesticide residues in food 2016

Special Session of the Joint FAO/WHO Meeting on Pesticide Residues

REPORT 2016

Glyphosate is unlikely to pose a carcinogenic risk to humans from exposure through the diet; glyphosate is unlikely to be genotoxic at anticipated dietary exposures
JMPR – June 2016

CONCLUSION ON PESTICIDES PEER REVIEW

EFSA Journal

APPROVED: 17 August 2017

doi: 10.2903/j.efsa.2017.4979

Peer review of the pesticide risk assessment of the potential endocrine disrupting properties of glyphosate

European Food Safety Authority (EFSA)

Abstract

EFSA was requested by the European Commission to consider information on potential endocrine activity of the pesticide active substance glyphosate in accordance with Article 31 of Regulation (EC) No 178/2002. In the context, the conclusions of EFSA following the peer review of the initial risk assessment carried out by the competent authority of the rapporteur Member State, Germany, are reported, following the submission and evaluation of pertinent data made available by the applicants. The current conclusion presents a follow-up assessment to the existing EFSA Conclusion on the peer review for the renewal of the approval of glyphosate (EFSA Journal 2015;13(11):4302) focussed on the outstanding issues identified in relation to the potential endocrine activity of glyphosate. The current assessment concluded that the weight of evidence indicates that glyphosate does not have endocrine disrupting properties through oestrogen, androgen, thyroid or steroidogenesis mode of action based on a comprehensive database available in the toxicology area. The available ecotox studies did not contradict this conclusion.

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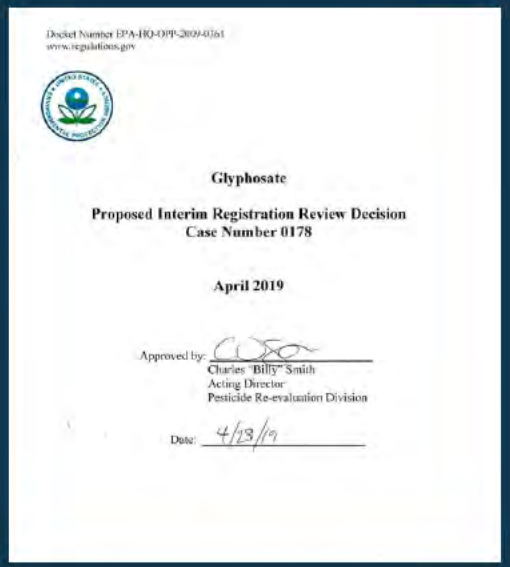
Keywords: glyphosate, peer review, potential endocrine activity, risk assessment, pesticide, herbicide

Requestor: European Commission
Question number: EFSA-Q-2016-00663
Correspondence: pesticides.peerreview@efsa.europa.eu

EFSA concluded that the weight of evidence indicates that glyphosate does not have endocrine disrupting properties based on a comprehensive database available in the toxicology area. The available ecotox studies did not contradict this conclusion.
EFSA – Approved August 2017



Docket Number EPA-HQ-OPP-2009-0361
www.regulations.gov

Glyphosate

Proposed Interim Registration Review Decision
Case Number 0178

April 2019

Approved by:
Charles "Billy" Smith
Acting Director
Pesticide Re-evaluation Division

Date: 4/13/19

The EPA conducted an independent evaluation of the carcinogenic potential of glyphosate and has determined that glyphosate is " not likely to be carcinogenic to humans."
EPA PID – April 2019

Safety Profile of Active Ingredient

Low Regulatory Risk based on EFSA 2015 conclusions

Low Risk
Medium; require refinements
High Risk

GTF 2

Endpoint	Regulatory Risk	Classification
Acute Toxicity		Irritating to eyes (a.i. only); **Cat. 1, R41; Classif. H318**
Developmental & Reproductive		Not a reproduction/developmental toxicant
Genotoxicity		Not genotoxic
Carcinogenicity		Not carcinogenic
Endocrine Disruption		Not an endocrine disruptor
Other		Not neurotoxic or immunotoxic

Outlined in the Renewal Assessment Report which supports the 2015 EFSA conclusions **(BfR, 2015)**

2001 Monograph relied on several data sets from registrants rather than on one ‘key study’

A1R 2 even more toxicology data; packages from:

- **Monsanto**
- **Cheminova**
- **Syngenta**
- **Arysta**
- **Feinchemie (Adama)**
- **Nufarm**

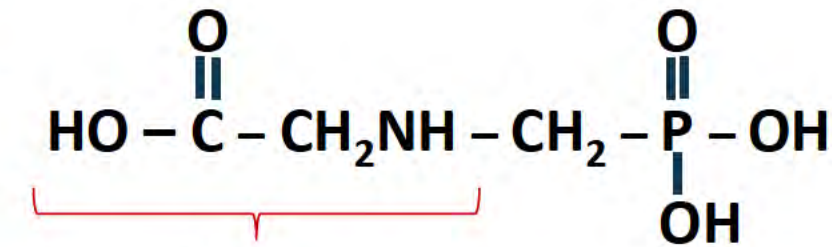
More than 900 scientific publications (published since 2000 until 2014) and other relevant information was considered

All these publication were assessed for relevance, quality and reliability and were used for risk assessment only on condition that the respective criteria had been met

The Kinetics of Glyphosate are Well Documented

This pattern of toxicokinetics and metabolism is independent of sex, dose level, or repeated administration **(EU, 2015)**

- Glyphosate is rapidly absorbed from the gut (oral absorption ~20%; mostly excreted unchanged in feces)
- Systemic glyphosate rapidly excreted via urine (within 48 hours; $T_{1/2}$ = 6-12 hours)
- Essentially no metabolism of absorbed glyphosate
- Shows no potential for bioaccumulation
- Very low dermal absorption; multiple formulations tested (human *in vitro* < 1% absorption)



Glycine conjugation is one type of
- phase II metabolism

Glyphosate *in vivo* behaves as
- conjugated methyl phosphonate

Therefore no surprise
- **No metabolism**
- **Rapid urinary excretion**
- **Polar - no bioaccumulation potential**

Acute Toxicity Studies

Glyphosate has low acute toxicity **(BfR, 2015)**

Study Type	Result†
Acute oral (rat & mice)	LD_{50} > 2000 mg/kg bw
Acute dermal (rat)	LD_{50} > 2000 mg/kg bw
Acute inhalation (rat)	LC_{50} > 5 mg/mL air
Eye irritation (rabbit)*	Severely irritating to the eyes (Annex VI CLP, H318)
Dermal irritation (rabbit)	Not irritating to rabbit skin
Dermal Sensitization (M&K, LLNA, Buehler)	Not a sensitizer

† Evaluated glyphosate acid and glyphosate salts

* Eye irritation properties of the glyphosate salts used in formulated products do not warrant their classification

Genotoxicity Studies

No evidence of a genotoxic potential in an adequate range of *in vitro* and *in vivo* studies **(BfR, 2015, RAR vol. 3)**

Study Type	Assays (# acceptable)	Results
in vitro	Ames (12; 4 supplementary) Mouse Lymphoma (2) HGPRT (1) Chromosomal Aberration (3; 1 supplementary) Unscheduled DNA synthesis (1) DNA Repair Test - Rec assay; (1 supplementary)	All Negative
in vivo	Micronucleus - MN (9) Chromosomal Aberration (2)	Negative 1 MN (F weak +ve @HDT, 5000 mg/kg)

Genotoxic potential of formulations

EFSA Conclusion (2015)
Conflicting EFSA guidance on genotoxicity assessment of chemical mixtures

"The peer review recognized that the issue of toxicity of the formulations should be considered further as some published genotoxicity studies (not according to GLP or to OECD guidelines) on formulations presented positive results *in vitro* and *in vivo*. In particular, it was considered that the genotoxic potential of formulations should be addressed"

If the assessment of all components of a chemically fully defined mixture results in the conclusion that none of these raises a concern with respect to genotoxicity, the mixture is also considered of no concern with respect to genotoxicity.

Conclusion from Genotoxicity Assessment of Chemical Mixtures (EFSA, 2019)

Question to AGG

- A considerable volume of genotoxicity data were generated for Art. 43 submissions which validates the EFSA guidance on mixtures
- Will the AGG align with the 2019 EFSA guidance genotoxicity assessment of chemical mixtures?

Genotoxicity studies for glyphosate-based formulations (GBFs) and formulation components

GBFs do not cause point (gene) mutations and are devoid of a clastogenic potential *in vivo* **(BfR, 2015, RAR vol. 3)**

Test Substance	Assays	Number of Studies	Results
Glyphosate Based Formulations	Ames	4	Negative
	Micronucleus (*in vivo*)	4	Negative
Surfactants	Ames	3	Negative
	Micronucleus (*in vitro*)	1	Negative
	Mam. cytogenetics	1	Negative

NOTE: Ongoing testing of formulations to support Art 43 submissions

Question to AGG

- Above studies were reviewed in A1R 2
- Does the AGG want GBF genotoxicity assays or formulation component genotoxicity assays submitted (other than for the representative formulation)?

Toxicity - Key Endpoints (A1R 2 List of Endpoints)

Glyphosate is not classified or proposed to be classified as carcinogenic or toxic for the reproduction **(BfR, 2015; ECHA, 2017)**

Species	Results (Overall NOAEL)	Conclusion
Reproductive	Parental & Offspring ~ 300 mg/kg bw/day Reproductive ~ 351 mg/kg bw/day	Reproductive performance was not altered in any study and glyphosate is unlikely to cause reproductive effects in humans.
Developmental	* 50 mg/kg bw/day (rabbit)	Glyphosate was not considered teratogenic in rats or rabbits.
Long-term	100 mg/kg bw/day	Glyphosate was considered unlikely to pose a carcinogenic risk in humans.

* Higher relevant NOAELs below driving LOAEL in other rabbit dev tox studies available

Evaluations	IARC	Experts & Regulators
Review Primary Study Report(s)	No	Yes
Consider Dose–Response	No	Yes
Consider Statistical Trend Analysis	Recalculated, positive. No trend if exclude doses ≥ limit dose. No trend across all studies	Yes [negative]
Review Pathology Report	No	Yes
Review Pathology Working Group Report	No	Yes
Consider histopathology of pre–neoplastic findings for treatment induced carcinogenesis	No	Yes no evidence of pre–neoplastic progression
Consider all studies; consistency across studies	No	Yes
Evaluate same neoplasm types across same/similar design in same species	e.g. hemangiosarcoma in one mouse study, renal neoplasms in another mouse study, but not across both studies for weight of evidence evaluation	(up to 6 mouse and 9 rat chronic/carc studies considered)
Consistent with Previous Expert Regulatory Reviews	No	Yes
Consistent with OECD Guidance Document 116 on Conduct & Design of Carcinogenicity Studies	No	Yes Evaluated statistics, biological plausibility, dose–response, pathology
Time	Days/weeks	Months/years

Endocrine Disruption

<u>Overall Conclusion</u>: glyphosate is not considered to be an endocrine disruptor

(BfR, 2017 – Glyphosate Addendum 2 to RAR)

Study Type	Assays (OECD Level)	Results
In vitro	ER Binding (2) ER Transcriptional Activation (2) AR Binding (2) Steroidogenesis H295R (2) Aromatase (2)	The weight of evidence indicates that glyphosate does not have potential to interact with the EATS-mediated endocrine pathways*
In vivo	Uterotrophic (3) Hershberger (3) Pubertal Male (4) Pubertal Female (4) Amphibian Metamorphosis (ECO) Fish Short-term Reproduction(ECO)	

* Revisit molecular structure and pharmaco-kinetics

Endocrine Disruption Conclusion

CONCLUSION ON PESTICIDES PEER REVIEW

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Question number: EFSA-Q-2016-00663

Correspondence: pesticides.peerreview@efsa.europa.eu

*The weight of evidence indicates that **glyphosate is not an ED.***
EFSA – September 2017

Submission will follow EFSA guidance on endocrine disruption assessment in Appendix E.

Endocrine Disruption – preparation of Appendix E

OECD Conceptual Framework			
Level 1	Existing data and new-nontesting information		
Level 2	In vitro assays on selected endocrine mechanisms	5 EDSP assays	Estrogen Receptor(ER) Binding Assay
			ERα Transcriptional Activation assay
			Androgen Receptor (AR) binding assay
			Steroidogenesis
			Aromatase Inhibition Assay
Level 3	In vivo assays on selected endocrine mechanisms	2 EDSP	Hershberger Assay
			Uterotropic assay
Level 4	In vivo assays on adverse selected selected endocrine mechanisms	2 EDSP	Pubertal developmental and Thyroid function in male rats
			Pubertal developmental and Thyroid function in female rats
		In vivo toxicity studies	70 studies with rats, mice, dogs and rabbits
Level 5	In vivo assay covering life cycle changes		6 Two-generational reproductive toxicity

Question to AGG

Given the significant quantity of higher tier data available, does the AGG consider Level 1 data unnecessary?

Amino**m**ethyl**p**hosphonic **A**cid (AMPA) – plant & soil metabolite

AMPA has a similar toxicological profile to glyphosate (EU, 2015)

Environmental metabolite and degradate of glyphosate

A number of toxicological studies are available on the metabolite AMPA

Overall it was concluded that AMPA presents a similar toxicological profile to glyphosate and the reference values of the latter apply to its metabolite AMPA.

ADI (BfR, 2015)

<u>Endpoint for the Acceptable Daily Intake (ADI)</u>:
Rabbit Developmental Toxicity (NUF, 1996)

- **ADI** of glyphosate = 0.5 mg/kg bw/day
- Based on the maternal and developmental NOAEL of 50 mg/kg bw per day from the developmental toxicity study in rabbits and applying a standard uncertainty factor (UF) of 100

Glyphosate Acceptable Daily Intake (ADI)
[redacted] (1996)

ADI
= 0.5 mg/kg bw/day

NOAEL =
50 mg/kg bw/day

LOAEL =
200 mg/kg bw/day
(severe maternal toxicity and post-implantation loss)

Supporting Rabbit Dev Tox Studies
- [redacted] (1980)
- [redacted] (1991)
- [redacted] (1996)
- [redacted] (1995)

GTF 2

ARfD (EFSA, 2015)

RAR (BfR, 2015)

ARfD: *Not established, not necessary*

<u>Endpoint for the acute reference dose (ARfD)</u>: Rabbit Developmental Toxicity (NUF, 1996)

- **ARfD** of glyphosate = 0.5 mg/kg bw/day
- Based on the same NOAEL of 50 mg/kg bw per day as the ADI (from the developmental toxicity in rabbits) due to the occurrence of severe maternal toxicity including mortality of pregnant does and the increased incidences of post-implantation loss observed in two of the seven developmental toxicity studies in rabbits, applying an UF of 100

Glyphosate Acute Reference Dose (ARfD)

[redacted] (1996)

ADI
= 0.5 mg/kg bw/day

NOAEL =
50 mg/kg bw/day

LOAEL =
200 mg/kg bw/day
(severe maternal toxicity and post-implantation loss)

Supporting Rabbit Dev Tox Studies

- [redacted] (1980)
- [redacted] (1991)
- [redacted] (1996)
- [redacted] (1995)

AOEL (BfR, 2015)

Endpoint for the acceptable operator exposure level (AOEL): Rabbit Developmental Toxicity (NUF, 1996)

- **AOEL** of glyphosate = is 0.1 mg/kg bw per day
- Same basis as the ADI and ARfD, applying a correction factor to account for the limited oral absorption of 20%
- Systemic exposures not consistent with high dose NOAELs in repeat dose dermal toxicicity studies

Glyphosate Acceptable Operator Exposure Level (AOEL)
[redacted] (1996)

ADI
= 0.5 mg/kg bw/day

NOAEL =
50 mg/kg bw/day

LOAEL =
200 mg/kg bw/day
(severe maternal toxicity and post-implantation loss)

Supporting Rabbit Dev Tox Studies
- [redacted] (1980)
- [redacted] (1991)
- [redacted] (1996)
- [redacted] (1995)

Rabbit developmental tox studies

Reliable Studies (RAR, rev. March 2015)

[redacted] (1996): Acceptable; 50, 200, 400 mg/kg/d

- Dosed GD days 7-19

[redacted] (1996): Acceptable; 100, 175, 300 mg/kg/d

- Dosed GD days 8-20

[redacted] (1995): Acceptable; 10, 100, 300 mg/kg/d

- Dosed GD days 6-18

[redacted] (1991): Acceptable; 50, 100, 450 mg/kg/d

- Dosed GD days 7-19

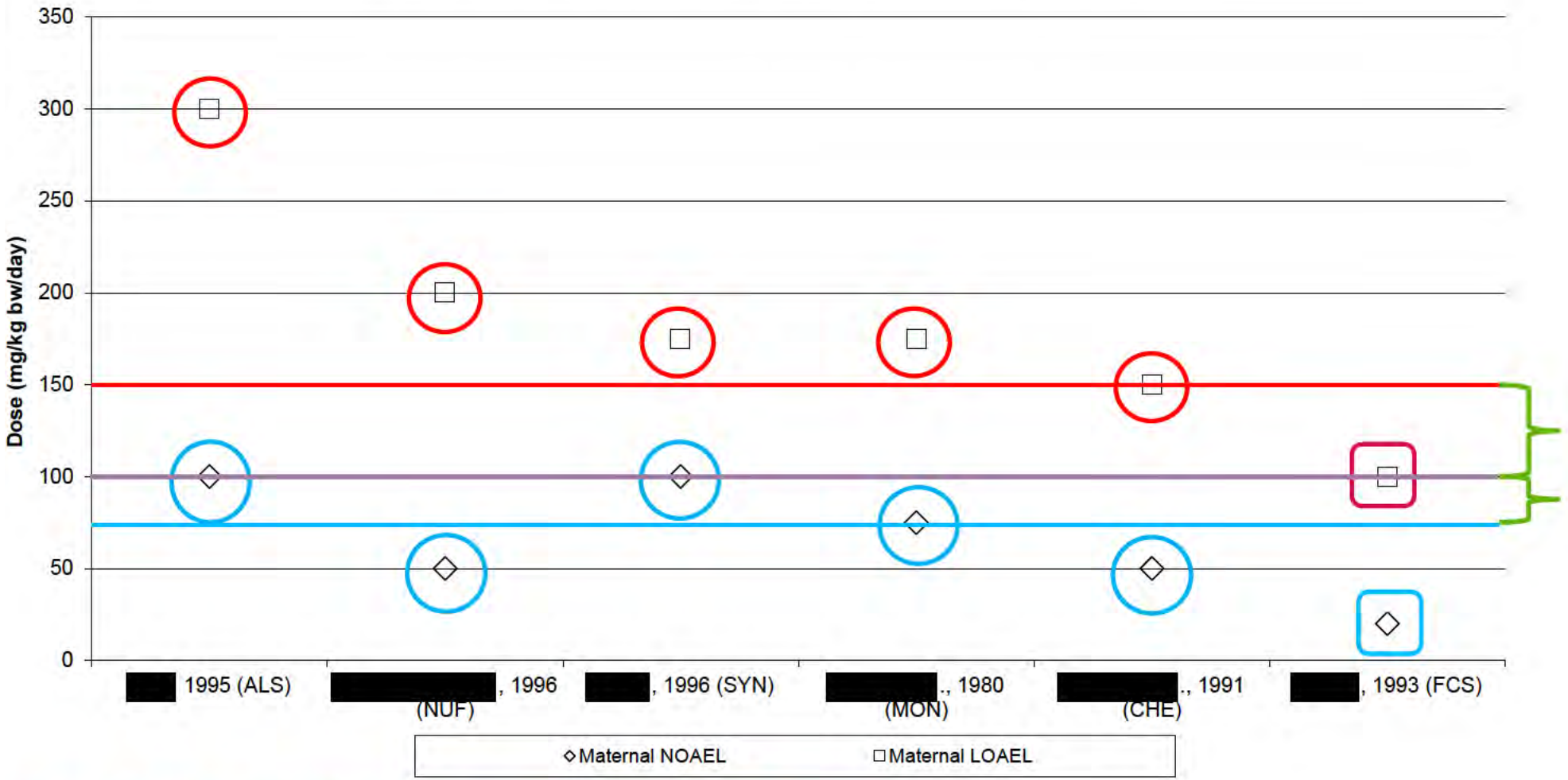
[redacted] (1980): Supplementary (high maternal mortality & ↓ to 6 litters @ high dose); 75, 175, 350 mg/kg/d

- Dosed GD days 6-27

[redacted] (1993): Supplementary; 20, 100, 500 mg/kg/d

- Dosed GD days 6-18

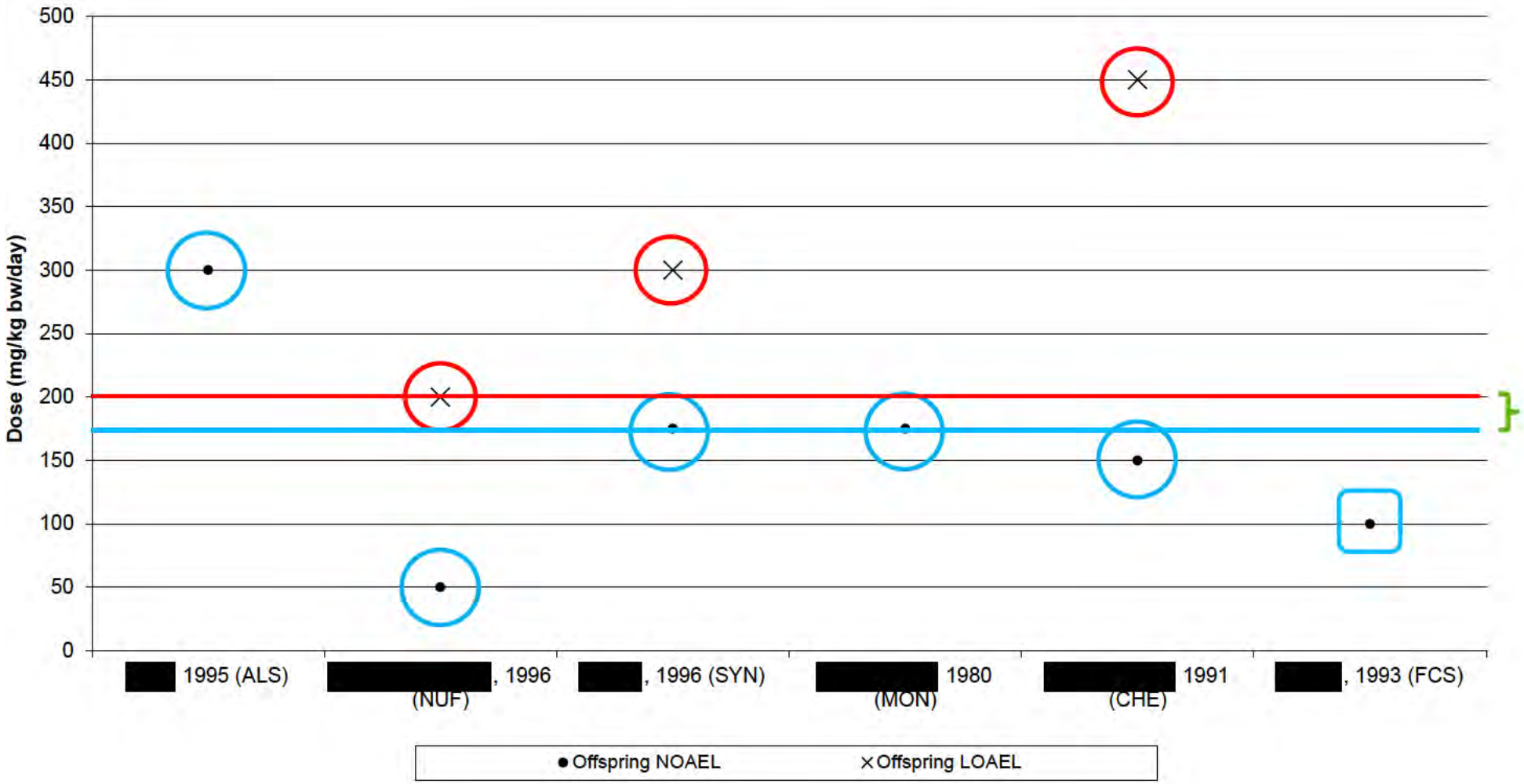
Rabbit Developmental Toxicity Studies – MATERNAL EFFECTS



All other studies considered more reliable than [redacted]

Overall WoE maternal NOAEL = 100 mg/kg/d or at least 75 mg/kg/d; LOAEL = 150 mg/kg/d

Rabbit Developmental Toxicity Studies Offspring Effects



Clear overall offspring NOAEL = 175 mg/kg/d; offspring LOAEL = 200 mg/kg/day

Rabbit developmental tox studies
Maternal toxicity or malnutrition?

Clinical signs included

- Soft/liquid stools & diarrhoea
- Reduced body weight and body weight gain
- Death
- Clinical signs & mortality most severe in [redacted] (1980) – dosed 21 days vs 12 days in other studies

Rabbits are coprophagic, requiring faeces consumption for nutrient recycling to maintain balanced nutrition

Coprophagy not feasible with soft stools & diarrhoea

- Therefore rabbits in developmental toxicity studies were nutritionally compromised
- Malnutrition explains severe mortality rate in last week of [redacted] (1980) high dose

  Mortality (high dose, 350 mg/kg/d): 5@ days 21-27 (all with diarrohea); 4@ days 17-20 (3/4 with diarrhoea)

  Diarrohea (high dose, 350 mg/kg/d): observed 20/27 rabbits, remaining animals with loose stools

Rabbit Nutrition

Lagomorphs, hind gut fermenting herbivorous small mammals (including rabbits), practice coprophagy (consume their own feces) in order to maintain balanced nutrition. This is a widely known fact and is documented in the scientific literature going back over 130 years;

“L'ingestion des crottes est un phénomène physiologique. Elle aurait pour but de soumettre les aliments à une seconde élaboration digestive et à une nouvelle absorption. **Cet acte est indispensable à l'entretien de la vie des léporidés**” (page 223 conclusions, Morat, 1882).

“Ingestion of droppings is a physiological phenomenon. It would aim to submit food to a second digestion and a new absorption. **This act is essential to the maintenance of life in rabbits and hares**” (English translation page 223 conclusions, Morat, 1882).

Morot MC (1882). Des Pelotes Stomacales des Léporidés. *Mem. Soc. Cent. Med. Vet.* 12 (1), 139-239.

Rabbit dermal repeat dose (sub-acute) toxicity studies EU Monograph (2001)

[redacted] (1982): NOAEL: 5000 mg/kg/d (HDT)

[redacted] (1994): NOAEL: 2000 mg/kg/d (HDT)

Determination of rabbit systemic NOAEL via dermal absorption through rabbit skin

[redacted] (2012): *in vitro* rabbit dermal absorption = 2.67% @ equivalent dosing to Johnson (1982) of 5000 mg/kg/d

Systemic dose for repeat dose dermal NOAEL of 5000 mg/kg/d

5000 x 2.67/100 = 133.5 mg/kg/day systemically available glyphosate

Equivalent oral dose to achieve 133.5 mg/kg/day systemic dose, based on oral absorption of 20%

133.5 /20% = **667.5 mg/kg/day oral dose…**

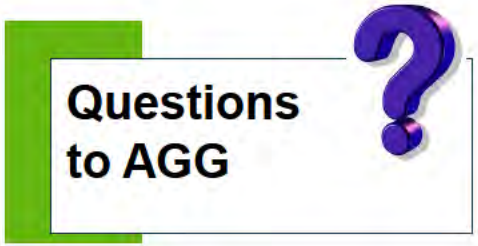
4 X higher than maternal effects in oral dev tox studies with a clear maternal LOAEL of 150 mg//kg/d

Rabbit developmental tox studies

Questions to AGG

- GTF submitted [redacted] (2012) in the A1R dossier with rationale for a systemic NOAEL, but study was isolate when placed in the dermal absorption section of the RAR…request consideration of this study for the intended purpose in upcoming 2020 submission in evaluation, showing rabbit systemic NOAEL greater than maternally toxic doses in rabbit dev tox studies
- AOEL based on maternal toxicity in rabbit
  - should be based on a systemic toxicity endpoint
- Is [redacted] (2012) together with [redacted] (1982) sufficient to infer that maternal toxicity in rabbit developmental toxicity studies is non-systemic?
- Given rationale considered by ECHA in RAC 40, not accepting proposed STOT RE2 classification based on rabbit dev tox maternal toxicity, does the AGG agree that rabbit inability to perform coprophagy resulted in compromised nutrition and mortality and was not a consequence of systemic toxicity?

Endpoint Selections



- Does the AGG agree that the rationale presented effectively shows that rabbit maternal toxicity was non-systemic and therefore different endpoint should be selected to calculate the AOEL?
- Since humans are not coprophagic, does the AGG agree that the rabbit is not an appropriate model to rely on for human health risk assessment when maternal toxicity is attributable to nutritional compromise?
- If so, does the AGG agree that the ADI and ARfD values should be based on different endpoints from the glyphosate data base?

EUROTOX 2019 Session 29: Species specific gastrointestinal (GI) toxicity in rabbits – what does it mean for prenatal developmental toxicity (PNDT) studies and their regulatory use?

Testing for prenatal developmental toxicity (PNDT) is a requirement under several regulations such as the REACH Regulation, the Biocidal Products Regulations (BPR) and the Regulation for the placing of plant protection products on the market (PPP). The preferred species are the rat and the rabbit and testing in two species is often required. However, rabbits are known to be susceptible to gastrointestinal (GI) imbalances. For example, antibiotics and poorly absorbed materials often disturb the gut microflora and/or cause diarrhoea and reduced food consumption, which can result in abortion, foetal resorption and maternal death. The results of rabbit PNDT studies showing GI imbalances may be of limited regulatory use. Hence, alternative testing should be considered. The first presentation of this session will provide the background on the physiology of the highly specific rabbit GI and will illustrate several problems that are arising for a Contract Research Organisation (CRO) when testing rabbits. The second presentation will address the limitations in the use of rabbit PNDT studies showing GI toxicity for plant-protection products (PPPs) from an industrial point of view. In the third presentation, the use of rabbits for pharmaceuticals will be high-lighted. Since rabbit is not a suitable species to test several antibiotics by the oral route, alternative modes of investigation and alternative species are available. The use of mouse and especially of mini-pigs as alternative species will be presented together with the latest development on study designs for testing mini-pigs. In the last presentation, the results of a scientific project will be presented by ECHA (European Chemicals Agency), in which results of 185 rabbit PNDT studies were analysed and a survey was performed among stakeholders that are performing and evaluating rabbit PNDT studies. Based on the results of the project, further needs will be high-lighted.

Gastrointestinal (GI) toxicity in rabbits – mechanisms and relevance for human
Manon Beekhuijzen, Charles River Laboratories, Den Bosch, Netherlands

Rabbit PNDT studies: What are the regulatory consequences for plant protection products?
Mary Moxon, MMTGS Limited, Congleton, United Kingdom

Alternative species and methods for embryo-fetal developmental toxicity studies for pharmaceuticals
Céline Pique, Charles River Laboratories, Lyon, France

Regulatory considerations for the evaluation of rabbit PNDT studies submitted under REACH and/or BPR
Ulrike Reuter, European Chemicals Agency, Helsinki, Finland

2016 JMPR WHO (~2004) evaluations

- Evaluated seven separate toxicology data sets (three sets in 2004)
  - // 3 data sets reviewed in 2004
- Endpoint: 2-year rat study salivary gland alterations
  - // Same study as PMRA for point of departure
  - // Higher NOAEL/LOAEL selected

"effects may be secondary to local irritation due to the low pH of glyphosate… unable to establish this unequivocally"

ADI = NOAEL/UF

= 100/100

= 1 mg/kg

LOAEL: 300 mg/kg bw/day

2019 US EPA Preliminary Interim Decision

- Evaluated internal data bases, open literature, publicly available information, additional submitted studies
- Revised Glyphosate Issue Paper: Evaluation of Carcinogenic Potential
  - *Not Likely to be Carcinogenic to Humans*
- Endpoint: Maternal toxicity in rabbit developmental toxicity study

ADI = NOAEL/UF

= 100/100

= 1 mg/kg

LOAEL: 175 mg/kg bw/day

2016 Japan FSC

- Evaluated five separate toxicology data sets (including “new” data set)
- Endpoint: highest relevant NOAELs across multiple studies below lowest LOAEL of same study designs
  - // 90-day rat
  - // 90-day and 1-year dog
  - // Developmental toxicity rabbit

ADI = NOAEL/UF

= 100/100

= 1 mg/kg

LOAEL: varied

2017 Canadian PMRA

- Evaluated multiple toxicology data sets
- Endpoint: 2-year rat study salivary gland alterations and ↓ body wt
- Not genotoxic; unlikely to pose a human cancer risk
- **JGTF opinion on salivary glands**
  - // Adaptive, non-adverse
  - // Proof of concept with citric acid dietary study
  - // Demonstrated reversible effects

ADI = NOAEL/UF

= 30/100

= 0.3 mg/kg

LOAEL: 100 mg/kg bw/day

Salivary Glands Adaptive Response to an Acidic Diet
Proof of Concept Study with Citric Acid – [redacted] (2010)
Incidence and Severity of Parotid Salivary Gland Acinar Cell Alterations

Dose	Control gavage	Low pH gavage	Control diet	Low pH diet	High pH diet
Parotid Salivary Glands [a]	9	10	10	10	10
Alteration, cytoplasmic	9	10	7	10	9
Incidence (%)	*100*	*100*	*70*	*100*	*90*
Minimal	8	6	5	0	4
Mild	1	3	2	6	5
Moderate	0	1	0	4	0
Average Severity [b]	1.1	1.5	0.9	2.4 *	1.4

[a] Number of tissues examined from each group.
[b] 1 = minimal, 2 = mild and 3 = moderate; no histologic change = 0
* Statistically Significant, p = 0.01, Mann-Whitney U-Test

8-week study

10 male rats/group

14,000 ppm citric acid

Gavage control adjusted to match weekly dietary intake 790-1300 mg/kg/day

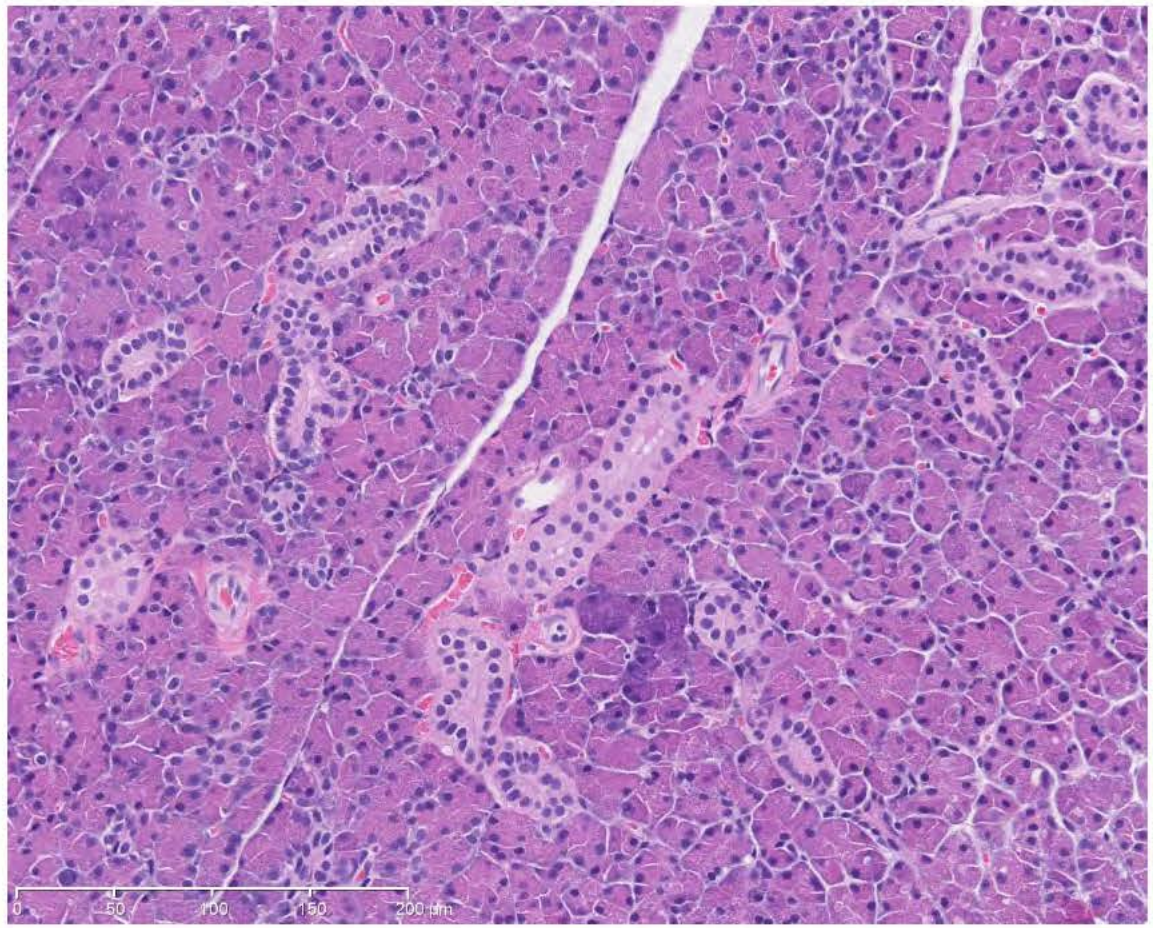
Stat sig. increased parotid salivary gland weights (absolute & relative weights)

Salivary Glands Adaptive Response to an Acidic Diet

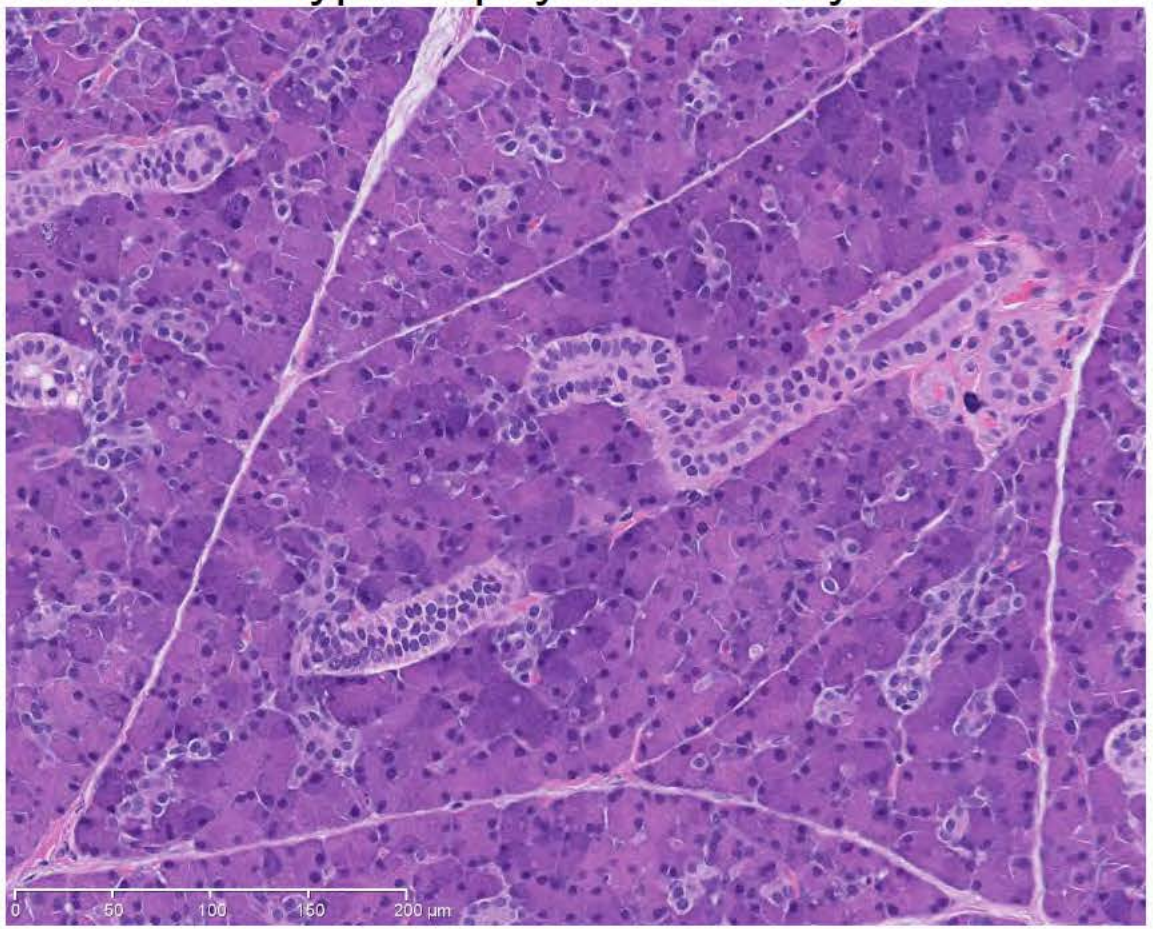
Proof of Concept Study with Citric Acid – [redacted] (2010)

Increased basophilic staining and hypertrophy of parotid salivary gland

Control diet



Acidic diet: hypertrophy of secretory acinar cells



No hyperplasia or cytotoxicity

HEALTHY ADAPTIVE RESPONSE

Alternative Endpoint Selections for ADIs

- JMPR (2004 & 2016): ADI = 1.0 mg/kg
  - Salivary gland effects weight of evidence (WoE) overall NOAEL = 100 mg/kg/d
- US EPA PID (2019): ADI = 1.0 mg/kg
  - Maternal toxicity in rabbit developmental toxicity overall NOAEL = 100 mg/kg/d
- Japan FSC (2016): ADI = 1.0 mg/kg
  - Overall NOEL across rabbit dev tox and repeat dose rat and dog
- Canadian PMRA (2017): ADI = 0.3 mg/kg
  - Salivary gland effects considered NOAEL = 30 mg/kg/d
- NOTE: all determined glyphosate
  - non-carcinogenic to humans
  - non-genotoxic in the most reliable studies using relevant routes of exposure

Toxicokinetic Study – data gap

To evaluate systemic exposure in combination with existing toxicokinetic data after dietary administration

- Rats (8/sex/dose) will be feed test diet for 14 consecutive days (2 doses, targeting 75 & 400 mg/kg/day) and then basal diet for up to 3 days during which toxicokinetic samples will be taken (0.5, 2, 5, 8, 14, 24, 36, & 48 hours)
- Bioanalytical samples will be analyzed for concentration of glyphosate and AMPA
- Parameters evaluated include;
  - t_{max} & t_{last} (last quantifiable concentration)
  - C_{max} & C_{max}/dose (normalized peak concentration)
  - AUC & AUC/dose (normalized daily exposure)

Comparative *in vitro* metabolism

Metabolic stability and profiling of [^{14}C]-Glyphosate for inter-species comparison.in hepatocytes from human, rat, mouse, dog and rabbit

Study in progress

Draft report expected 4Q 2019

Groups	Components added			Purposes	Acceptability criteria
	Test Item	Buffer	Cryo-preserved primary Hepatocytes		
Species	x	x	x	Determine full reaction	-
Control 1	x	x	-	Determine stability of the test item in buffer	Degradation of test item is less or equal to 10 % after 120 min
Control 2	x	x	x	Determine functionality of the test system: metabolic activity test of phase I Conversion of Testosterone to 6β- Hydroxytestosterone	Degradation of Testosterone is more or equal to 80 % after 120 min
Control 3	x	x	x	Determine functionality of the test system: metabolic activity test of phase II Conversion of 7-Hydroxycoumarin to 7-Hydroxycoumarin glucuronide as well as 7-Hydroxycoumarin sulfate	Degradation of 7-Hydroxycoumarin is more or equal to 80 % after 120 min
Control 4	x	x	inactivated hepatocytes	Determine if reaction is hepatocyte dependent	Less or equal to 2 % active cells are observed under the microscope

Phototoxicity and Photomutagenicity (photoreactivity)

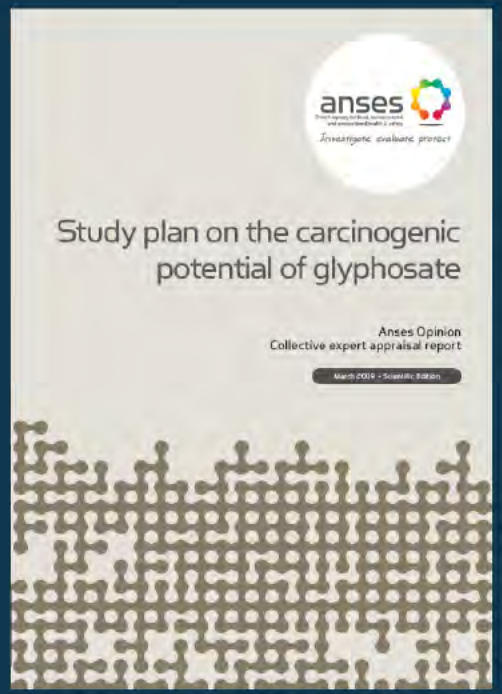
If the UV/visible molar extinction coefficient (molar absorptivity) of the active substance is less than 10 $L.mol^{-1}.cm^{-1}$, no phototoxicity testing is required.

- Studies triggered depending on the value of ε (molar extinction coefficient $L.mol^{-1}.cm^{-1}$) of the chemical in aqueous solution at the wavelength of maximum absorption in the UV/visible spectrum (λ_{max})
  - If ε is ≥ 10 $L.mol^{-1}.cm^{-1}$ → phototoxicity test (OECD 432, 2004)
  - If ε is ≥ 1000 $L.mol^{-1}.cm^{-1}$ → photoreactivity test (OECD 495, 2019)
- RAR Annex Point B.2.5.1.5. (IIA 2.5.1): OECD 101 UV/visible absorption spectra available

Question to AGG

- Data previously accepted for A1R do not trigger the need for further testing following OECD 432 or 495
- Will the AGG please confirm this meets the requirements for phototoxicity and photomutagenicity (photoreactivity) evaluation?

ANSES – Study plan on the carcinogenic potential of glyphosate



ANSES Opinion Collective expert appraisal report March 2019 - Scientific Edition

Recommended Studies

- In vitro tests to study cellular stress following exposure to glyphosate
  - An in vivo comet assay combined with a micronucleus assay
  - A cell transformation assay combined with the transformics method

Question to AGG

// How will these studies be handled in the course of the renewal evaluation?

Thank You!

Genetox update for representative formulation MON 52276

Based on received questions from Anses for other formulations under Article 43

Micronucleus (in vitro) – Acceptability in question

- Lower limit of the positive control HC range is in the upper limit of the negative control HC range
  - (1) Positive controls should be weakly positive to demonstrate the sensitivity of the assay to detect low level positive responses; (2) The TG does not specify that the HC ranges must not overlap; (3) Having a weak positive control ensures that the blinded score is not biased; (4) When HC data is focused on the specific sex and vehicle used in this specific study, there is no overlap of the ranges.
- High dose selection is questioned
  - The formulation has a fully defined composition, therefore the limit dose is justified. The limit dose provides adequate sensitivity to detect mutagenic effects of test materials. EFSA's statement on Genotoxicity assessment of chemical mixtures (2018) supports this choice of doses.
- No analysis performed on test substance dose formulations
  - The test substance dose formulations were prepared freshly just before use and the lack of stability, homogeneity and concentration have no adverse impact on the validity of the study

Ames – Acceptable with limitations

- Some overlap in maximum negative control and minimum positive control values in the HC data
  - No overlap in the 95% control limits
- High dose selection is questioned: See response above
- No analysis performed on test substance dose formulations: See response above