Glyphosate Task Force: Comments on draft Opinion proposing harmonised classification and labelling at EU Level of glyphosate

1. Human Health Hazard Evaluation

The GTF supports the human health hazard evaluation and classification proposal in the draft opinion. In our comments we provide additional complementary details and nuances to make some arguments more robust and/or compelling.

1.1 RAC evaluation of skin corrotion/irritation

Page	*Text fragment*	*Comment*
9	Thus, there is insufficient human data to support classification.	There is insufficient evidence of skin effects in humans that would lead to classification

1.2 RAC evaluation of Respiratory sensitization:

Page	*Text fragment*	*Comment*
12	Since no data was provided and therefore no classification proposal was presented for this hazard class in the CLH report, it was not assessed by RAC.	The reason why such a test was not conducted was that there was no trigger for it • No structural alerts • No incidence during history of use

1.3 RAC evaluation of specific target organ toxicity-repeated exposure (STOT RE)

Comments regarding the discussion of the rabbit maternal toxicity in developmental toxicity studies:

Page	*Text fragment*	*Comment*
16	However, rabbits seem to be a much more sensitive species for effects arising from glyphosate exposure.	Via the oral route in regards effects on the functioning of the gastro-intestinal system (local effects rather than systemic effects)
18	However, decreased food consumption and reduced bw gain were also reported in does without premature death at similar doses of glyphosate as administered in the studies with premature death. Therefore, the premature death reported is not considered to be only related to decreased food consumption and reduced bw gain.	Up to and including the dose level of 300 mg/kg bw several rabbits died from the consequences of entry of test material in the respiratory tract: - 1 at 0 mg/kg bw and 1 at 200 mg/kg bw (Coles and Doleman, 1996) - 1 at 75 mg/kg bw (Tasker et al., 1980) - 2 at 0 mg/kg bw, 1 at 100 mg/kg bw (Suresh, 1993)

		In the best conducted and best reported study (Moxon, 1996) all rabbits that had to be killed suffered from loss of body weight and a decrease in food consumption
18	Soft/liquid stool and diarrhoea was also a consistent feature reported in most of the rabbit developmental toxicity studies indicating a local irritating effect of glyphosate in the gastrointestinal tract.	Other local effects of glyphosate in the rabbit GIT are: - Osmotic effect (watery content of caecum) - Stasis of GIT motility (hairballs) - GIT overload of test material (low absorption rate)
18	However, a clear association between the premature maternal death and soft/liquid stool and diarrhoea cannot be established.	In the best conducted and best reported study (Moxon, 1996) all rabbits that had to be killed suffered from few faeces and diarrhea. There were no cases of mal-gavage in this study
19	However, studies of rabbits completely deprived of caecotrophs demonstrate that while caecotrophy is very important for normal growth, it is not always essential for survival (Robinson et al., 1985, Phiny et al., 2006).	The reason why rabbits were found dead or had to be killed (excluding those suffering from the consequences of mal-gavage and regurgitation) has not to be fully ascribed to the lack of access to caecotrophs. GIT stasis (causing nausea and stress) altered caecal pH and dysbiosis (*Clostridium*) leading to enterotoxemia may have been contributory factors
20	However, only two studies may be considered as appropriate for consideration for STOT RE 2 but there remain some uncertainties related to the cause of the premature maternal deaths in the studies by Suresh et al., (1993) and Tasker et al., (1980).	The Tasker *et al.* (1980) study can be dismissed because all the animals suffered from soft stools and diarrhea indicating that there was a problem with the rabbit feed (too low fibre content?) causing gastro-intestinal disturbance. The high incidence of mal-gavage, the high rate of mortality (53% at 500 mg/kg bw) and the very poor reporting makes the Suresh et al. (1993) study invalid for STOT-RE classification.
21	Conclusion	GTF is in total agreement with this conclusion

1.4 RAC evaluation of germ cell mutagenicity

Page	***Text fragment***	***Comment***
28	In conclusion, the in vitro and in vivo data suggest that glyphosate may induce oxidative stress. However, increased levels of oxidative stress was not reliably demonstrated in the repeated dose studies where this was examined.	There is no strong weight-of-evidence that glyphosate acid as such produces oxidative damage to DNA in vivo since most of the studies have been conducted with glyphosate-based formulations and/or used irrelevant doses and/or routes of exposure (IP).

		There is no evidence of oxidative damage of DNA caused by glyphosate when administered via the oral route.

1.5 RAC evaluation of carcinogenicity

Page	***Text fragment***	***Comment***
32	*Pancreatic islet cell tumours in the rat:* However, the DS also noted a statistically significant positive trend for carcinomas in male animals in the Lankas et al (1981) study, which had not been previously reported.	The findings in the Lankas et al (1981) study don't make any sense since these were observed at a dose range that is 30-fold less than that of the Stout and Ruecker (1990) study
32	*Pancreatic islet cell tumours in the rat:* There was no incidences of pancreatic tumours in the females. No dose-response relationship was observed and there was no indication of progression to malignant neoplasia in either study. The DS also noted that an increased incidence of pancreatic tumours was not reproducible in other, more recent and OECD TG-compliant studies, …	There was also no dose-response relationship of pre-neoplastic lesions
33	*Malignant lymphoma in the mouse:* In the studies by Wood et al. (2009) and Sugimoto (1997), the findings were statistically significant when the trend test was applied, but not when a pairwise comparison was performed. The increased incidence in the study of Kumar (2001) was not confirmed neither by the trend test nor by a different pairwise test but only using the Z-test which had been used in the original study report.	Wood et al. (2009): There is indeed a positive trend in this test but the incidence at the highest dose level (10% at 810 mg/kg bw) is still within the historical control range of the laboratory (0-16%). The 0% in the control group is unusual for malignant lymphoma in male mice. Sugimoto (1997): There is indeed a positive trend in this test but the trend is positive because of the increase in incidence above the controls at an exceedingly high dose (4348 mg/kg bw!) and still that incidence (12% for all animals) was within the historical control range of the laboratory (4-19%). Kumar (2001): An independent statistical re-analysis of all the raw pathology data (non-neoplastic and neoplastic) carried out by [redacted] (AnaPath GmbH report, 2017) demonstrated that there is no statistically significant increase (trend test and pair-wise comparison) in the incidence of malignant lymphoma in Swiss albino mice treated with Glyphosate Technical at doses up to 10,000 ppm for a duration of 18 months
39	Table 6: Incidences of renal adenomas and carcinomas combined in male mice	In this table, the p-values presented are calculated using the approximate trend test

		which is inappropriate for this type of data (not sufficient number of animals with tumors, doses not evenly spaced) since they produce an exaggerated significance. The use of exact p-values is the appropriate approach: Knezevich, 1983: p=0.065 Sugimoto, 1997: p=0.062 Kumar, 2001: p=0.063 Atkinson, 1993: p=0.98 Based on this calculation none of the studies shows a significant positive trend for renal tumors. Also in the tables on malignant lymphoma and haemangiosarcoma approximate p-values were calculated instead of exact p-values A report detailing tehse statistical considerations ([redacted] 2017) is available upon request. Although the corrected p-values do not change the rapporteur's conclusion but should be considered for overall accuracy and quality of the assessment.
52	RAC notes that a tendency of increasing tumour incidences in male mice of the high dose groups is suggested across the studies available.	It is unclear what is meant by this statement: *Renal tumors:* when the exact trend test is applied the trend is not significative *Malignant lymphoma:* when the exact trend test is applied, the trend is only significative for the Sugimoto, 1997 study (p=0.02) and the Wood, 2009 study (p=0.008). The incidence at the high dose in both studies is still within the historical control range and the trend in the Sugimoto study is positive (p=0.02) because of the only increased incidence at 4348 mg/kg bw. The other 3 mouse studies didn't show a positive trend (lack of consistency). *Haemangiosarcoma*: when the exact trend test is applied the trend is only significative for the Atkinson, 1993 study (p=0.004). The other 4 mouse studies didn't show a positive trend (lack of consistency).
34		GTF agrees with the DS that no classification of glyphosate as carcinogenic is warranted

1.6 RAC evaluation of reproductive toxicity

Comments regarding embryo-fetal developmental toxicity in the rabbit:

68	Hojo (1995): … a statistically significant increase in the numbers of litters (%) with malformations were reported. The litter incidences were 1(5.6 %), 3 (20 %), 3 (18.8 %) and 5* (35.7 %) from the 0, 10, 100 and 300 mg/kg bw/d dose groups, …	When the malformations are split into external, visceral and skeletal malformations and visceral and skeletal variations only the total number of fetuses with skeletal variations was statistically significantly elevated at the mid-dose
68	Hojo (1995): The increases in malformations were mostly related to an increase in skeletal malformations including fusion of the frontal/partial bones, hypoplasia of the interparietal bone, shortening of the nasal/frontal mandibular bones and hemivertebra.	The number of fetuses with skeletal malformations was elevated at all dose levels without statistical significance and without any dose-response relationship. When the skeletal malformations are split into each individual anomaly then there is no dose-response relationship for any of them
72		GTF agrees with the DS that no classification of glyphosate as toxic to reproduction (development) is warranted

2. Environmental hazard

Summary

In accordance with the classification categories in Table 4.1.0 (b) of Regulation (EC) No 1272/2008, glyphosate does not meet the environmental classification criteria of a "Long-term (chronic) aquatic hazard, Category 2". This conclusion is based on robust and scientifically valid long-term (chronic) aquatic toxicity data available for organisms for different aquatic taxa groups.

The former environmental classification of glyphosate was based on a marine algae study which has been dismissed as invalid in the review of glyphosate under Annex I Renewal. The current CLH environmental classification proposal for glyphosate "Long-term (chronic) aquatic hazard, Category 2" (CLH report V.1, 2016, section 5.4.1.2, p. 107-108) has been based on an invalid zebra fish (Dias Correa Tavares, 2000) short term toxicity assay, which does not meet the requirements for being a long-term (chronic) early-life stage (ELS) assay (OECD TG 210) and **fails the validity criteria for being a reliable toxicity test** (specific comments on the study and the CLH classification are presented in the comments section below.)

The available valid and scientifically robust long term fish toxicity studies have not been considered in the classification proposal. These include a 255-day fish full life-cycle (FFLC) study conducted with *Pimephales promelas*, that achieved a NOEC of >25.7 mg/L based on no effects on growth, development, survival, and reproduction. In an **85-day full fish early life-stage (ELS) study** conducted with *Oncorhynchus mykiss*, a **NOEC of 9.6 mg/L** was achieved, based on no effects on growth, development and survival.

If despite the various deficiencies observed the zebra fish study (Tavares, 2000) is deemed acceptable for chronic classification, the values used for the classification should be based on **statistically relevant** findings to ensure reliability and relevance of the observed effects. In the proposed environmental classification, the NOEC value of 1 mg/L set by the evaluator, is based solely on **biological observations**, and does not consider the statistical significance of the findings. Considering the raw data from the short-term zebrafish study, a NOEC value of 3.2 mg/L would be applicable, as clearly stated by the author in the study report. In addition, if calculated, an **EC_{10} value of 3.48 mg glyphosate /L** would be achieved, which in common with the statistically relevant and scientifically robust chronic fish toxicity endpoints, is outside of the classification criteria required of a "Long-term (chronic) aquatic hazard" (comments 2 and 3, below).

Comments on the draft RAC Opinion report and the Validity of the Tavares (2000) Zebra fish toxicity study

Comment 1: Page 76-77, Summary of relevant information on aquatic toxicity

Page 77 of the draft opinion (2017) states that "*for each test all validity criteria per OECD guidelines were fulfilled and the studies are considered to be acceptable and valid*". This is correct for all studies except the zebra fish study (Dias Correa Tavares, 2000), which is the study relied upon to classify glyphosate.

Table 2: Validity criteria in zebra fish short-term study (Tavares, 2000)

OECD validity criterion	Study parameter	Observation
Mortality in controls < 10 %	0%	Validity criterion met
dissolved oxygen concentration from 60 – 100 % throughout the test	60-100%	Validity criterion met
Water temperature throughout the test should be constant (± 1.5 °C)	**Acclimation: 28 °C Test: 23.8-24.3**	**Validity criterion not met!**

In addition to the essential validity criteria listed above, the guidelines OECD 210 and OECD 212 recommend / require parameters to be followed, which impact the quality and reliability of the study. These are listed in Table 3.

Table 3: Other quality criteria in zebra fish short-term study (Tavares, 2000)

Recommendations / Requirements	Study parameter	Observation
Exposure at early gastrula stage (< 30 min after fertilization)	**48 hours post fertilization**	**Criterion not met!**
Termination at 5 days' post hatch	**Termination 7 days post hatch**	**Criterion not met!**
Water hardness 250 mg/L $CaCO_3$	**25 - 44.1 mg/L $CaCO_3$**	**Criterion not met!**
pH recommended 7.8 ± 0.5	**7.0 - 7.2**	**Criterion not met!**
Test media concentration measured	**Only stock solution measured**	**Criterion not met!**

Therefore, the endpoint from the study by Tavares (2000) should be **excluded from the list of reliable endpoints for long-term (chronic) toxicity to fish**.

Comment 2: The NOEC derived in draft RAC opinion (2017) is not "*the test concentration immediately below the lowest tested concentration with statistically significant adverse effect*".

The NOEC, i.e. *"the test concentration immediately below the lowest tested concentration with statistically significant adverse effect"* and thus the test concentration without a *"statistically significant adverse effect compared to the control*" as defined by Regulation (EC) No 1272/2008, amounts to **3.2 mg a.s./L** in the

study by Tavares (2000). This is clearly above classification criteria for long-term aquatic hazard of non-rapidly degradable substances for which there are adequate chronic toxicity data available.

Comment 3: Preferential use of EC_{10} over NOEC for conclusions on classification

Based on 4.1.2.7.2. of Regulation (EC) No 1272/2008: "***The NOECs or other equivalent EC_x (e.g. EC10) shall be used***."

Applying the worst-case among the various calculation models, i.e. the log-logistic distribution, an EC_{10} of 3.48 mg/L glyphosate would be predicted, which is consistent with the empirical data.

Conclusions of comments 1-3: In sum, the study by Tavares (2000) is rated **unreliable** and should not be considered for classification under Regulation (EC) No 1272/2008. The **85-day ELS** should be relied upon for chronic toxicity to fish, with a value of **NOEC=9.6 mg/L**. However, if the Tavares study is considered for classification (despite its unreliability due to various significant deficiencies), the statistically relevant NOEC of 3.2 or the respective EC_{10} of 3.48 value should be the endpoint in accordance with current ECHA guidance and Regulation (EC) No 1272/2008.