From: [redacted]@aces.su.se>
Sent: jeudi 14 septembre 2023 17:04
To: [redacted] (SANTE); [redacted] (EFSA)
Cc: [redacted]
Subject: glyphosate/DNT

Dear [redacted] dear [redacted]

we are reaching out to share concerns that the recent EFSA Conclusions regarding glyphosate may not be protective for human health with respect to developmental neurotoxicity, DNT.

We have read EFSA's conclusions for glyphosate from July 2023 as well as sections relevant to DNT from the Peer Review Report and the updated RAR from February 2023.

It is encouraging to see that EFSA and RMS have put substantial effort into evaluating DNT-related evidence, despite the fact that this issue unfolded at a late stage of the glyphosate evaluation process. We recognise the difficulties of evaluating DNT in the face of a constant stream of new academic studies.

We agree with EFSA's conclusion that the available data do not convincingly demonstrate that glyphosate causes DNT.

We do not agree with the conclusion that neither a DNT study nor an extra assessment factor is necessary. In our opinion, the indications of DNT are sufficiently strong to merit a prompt investigation by means of a guideline DNT study.

In particular:

- According to the RAR and the EFSA conclusions, a guideline DNT study is not needed based on a lack of neurotoxicity in adult animals in the available regulatory studies. We do not agree with this reasoning:
  The presence or absence of adult neurotoxicity has been used for prioritising

compounds for resource-intensive in vivo DNT testing, considering that neurotoxic compounds can be suspected of also causing DNT. However, adult neurotoxicity is not a prerequisite for DNT. There are numerous processes active in the developing brain that are not or hardly active in the mature brain, and effects on these processes cannot generally be probed by studies in adult animals. Most of the processes included in the proposed OECD in vitro DNT battery[1] fall into this category (e.g. cell migration processes). **In cases of direct indications of DNT** e.g. from academic studies (see below), **an absence of neurotoxicity in adult animals cannot be used to resolve DNT concerns**. For example, the compound etofenprox did not show any neurotoxicity in two adult neurotoxicity studies in the regulatory dataset, but affected behavioural functions in a DNT study[2] and also showed activity in several in vitro assays from the DNT screening battery[3].

- Regarding reproductive toxicity (comprising DNT), the data requirements on Regulation 283/2013 prescribe that
  "*Potential neurotoxic … effects … shall be carefully addressed and reported*."
  and
  "Investigations shall take account of all available and relevant data, including … knowledge concerning structural analogues to the active substance."
  Guideline DNT studies of the compounds glyphosate-trimesium[4] and glufosinate-ammonium[5], both available within the applicant group, have shown DNT effects. Both compounds may be regarded as structural analogues of glyphosate acid in so far as they are phosphonates and alpha-amino acid derivatives. It is possible that these existing DNT studies would be sufficient to trigger a DNT study for glyphosate acid. **A discussion about the DNT observed in structural analogues is lacking in the dossier and in the RAR**[6].
- The EFSA conclusions state an absence of activity of glyphosate in all the in vitro DNT assays included in the CompTox database. This is true, but it must be kept in mind that glyphosate has only been tested in 7 out of 17 available DNT assays[7]. For the brain developmental processes of cell migration, neuronal differentiation, glial differentiation, and myelination, glyphosate has not been tested in any of the available assays.
  For example, glufosinate-ammonium showed activity in the glia migration assay NPC2a[8]. Glyphosate has not been tested in this or any other cell migration assay.
  An absence of an effect on *some* neurodevelopmental processes should not be confused with an absence of an effect on *all* neurodevelopmental processes in in vitro assays.
- We fear that the proposed reference values may not be sufficiently protective. If the "worst case" proposed by one member state[9] was true and glyphosate acid would show the same effects as glyphosate trimesium in a DNT study, the ADI and ARfD would likely be lowered by factors of 5 and 15, respectively, compared to the values proposed in the EFSA conclusions[10]. **The conclusion that the proposed reference values are protective of DNT seems not well supported**.
- Academic literature: Three published articles reporting DNT-related endpoints after developmental glyphosate exposure in rodents are included in the RAR; glyphosate was used in one of the forms that are currently under assessment[11]: [redacted]2020[12],

2021[13], and 2023[14]. 2020 identified effects on motor activity and cognitive functions; 2021 identified effects on cognitive functions but did not assess motor activity. 2023 did not identify effects on brain weight and hippocampal morphology but on neurogenesis; behavioural functions were not addressed in this work. 2023 was referred to in the EFSA Conclusions as "DNT-related endpoints were assessed and considered as not affected"[15]. We would like to stress that there is no contradiction between these three studies with respect to behavioural effects. In addition, we note that several (although not all) academic studies on behavioural effects of developmental glyphosate-based herbicides (GBH) exposure included in the RAR, report effects on the same behavioural domains, which also applies to the guideline DNT study on glyphosate trimesium.
The study designs are very diverse, and it appears difficult to integrate this evidence. **Results from the academic literature however seem to align sufficiently well to support an overall indication of DNT.**

The EFSA WG expressed their opinion regarding DNT of glyphosate, written before the expert meeting: "*Overall, the EFSA WG is of the opinion that the current dataset for glyphosate is not suitable/sufficient to conclude on DNT hazard. (…) in the studies conducted with alternative glyphosate salts or with formulated products, there is indication of effects on DNT endpoints (…) the potential of glyphosate active substance of affecting in vivo DNT endpoints cannot be concluded and that an additional uncertainty factor might be considered to cover this data gap.*" [16]

We agree with the assessment of the evidence expressed in this opinion by the EFSA WG. From our perspective, the insufficiency of the dataset for definitive conclusions, the observed DNT indications in a diverse set of studies, and the fact that DNT is a property that is generally of high concern, warrant a prompt resolution of this issue, likely in the form of an in vivo DNT study according to OECD TG 426. The study design should address the precise endpoints affected in 2020 and 2021. In addition, the study should address behaviours[17] that have been affected by developmental exposure in studies of GBH: social behaviours (Ait-Bali 2020[18], Del Castilo 2022[19]), depression-like behaviours (Cattani 2017[20], Ait-Bali 2017[21]), and behaviours resembling autism spectrum disorders (Pu 2020[22]).

In the short term, an extra assessment factor or a conservative application of results from glyphosate-trimesium to other forms of glyphosate may provide the required high level of protection for human health.

In addition, we would like to highlight that the RAR and the EFSA Conclusions state that a guideline DNT study for glyphosate is not necessary. We also know that in the past, companies have withheld existing DNT studies from EFSA for some compounds[23], in some cases arguing that such studies have not been requested by EFSA[24]. We suggest that the Commission should ensure that applicants have received a formal request to submit any existing guideline DNT study of glyphosate or any knowledge of such a study, as well as any other information potentially indicating DNT[25].

With kind regards

and

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[1] OECD 2023 Initial Recommendations on Evaluation of Data from the Developmental Neurotoxicity (DNT) In-Vitro Testing Battery. https://www.oecd.org/env/ehs/testing/developmental-neurotoxicity.htm accessed 2023-09-07

[2] Two NT studies were included in the 2019 Dossier: (1) acute oral neurotoxicity study in rats, 2002, OECD TG 424, dose levels 0, 25, 125, 500, or 2000 mg a.s./kg bw/day. (2) 13-week dietary neurotoxicity study in rats, 2003, OECD TG 424, mean achieved dose levels 0, 149, 299 and 604 mg a.s./kg bw/day (males) and 0, 174, 350 and 690 mg a.s./kg bw/day (females). No neurotoxic effects were reported at any dose level in these two studies. One DNT study was included: mean achieved dose levels 0, 28.4, 79.2 and 238 mg a.s./kg bw/day. The dossier reports a NOAEL for functional development of 79 mg a.s./kg bw/day based on behavioural effects at the highest dose level.

[3] https://comptox.epa.gov/dashboard/chemical/concentration-response-data/DTXSID9032610 accessed 2023-09-07

[4] Documentation available to us is slightly equivocal regarding EFSA's overall conclusions from the guideline DNT study of glyphosate trimesium. It appears that experts concluded that motor activity was affected at the top two dose levels, and possibly learning and memory at the highest dose level (for males).

[5] U.S. EPA concluded regarding this study brain morphometric changes at all tested dose levels, and also identified behavioural effects (motor activity) at several time points. Accessed from https://www.regulations.gov/document/EPA-HQ-OPP-2016-0093-0183 on 2023-09-07

[6] We agree that the toxicological profile of glyphosate trimesium differs from the profile of glyphosate acid, which is a point that has been made several times in the RAR and Peer Review Report. We see however no explicit appreciation that the observed effects in the DNT study of glyphosate trimesium *could* have been caused by the glyphosate moiety.

[7] This is also mentioned in Annex 8 to TC 80 in the Peer Review Report, p. 201 of 255

[8] https://comptox.epa.gov/dashboard/chemical/concentration-response-data/DTXSID1024120 accessed 2023-09-13
[9] Peer Review Report Part 3, Expert meeting report. Part 3_Peer Review Report_Glyphosate_expert meeting reports_public.pdf, p. 211 of 672
[10] Assuming a NOAEL for behavioral effects (motor activity) of 10 mg/kg bw/day as the basis for ADI and ARfD and an assessment factor of 100
[11] According to RAR Volume 1 Section 1.3.1, the active substance is glyphosate acid and its isopropyl-amine, potassium, ammonium, and dimethylammonium salts
[12] https://pubmed.ncbi.nlm.nih.gov/32805371/
[13] https://pubmed.ncbi.nlm.nih.gov/33837468/
[14] https://pubmed.ncbi.nlm.nih.gov/36332718/
[15] EFSA conclusion on glyphosate, 2023 p. 12: "*an in vivo study in rats where DNT-related endpoints were assessed and considered as not affected by the high doses*".
[16] Peer Review Report Part 3, Part 3_Peer Review Report_Glyphosate_expert meeting reports_public.pdf, p. 208 + 209 of 672.
[17] OECD TG 426 offers the flexibility to include additiobnal behaviours in points 6 and 13.
[18] https://pubmed.ncbi.nlm.nih.gov/32067069/
[19] https://pubmed.ncbi.nlm.nih.gov/35628394/
[20] https://pubmed.ncbi.nlm.nih.gov/28627408/
[21] https://pubmed.ncbi.nlm.nih.gov/28848410/
[22] https://pubmed.ncbi.nlm.nih.gov/32398374/
[23] https://pubmed.ncbi.nlm.nih.gov/37259092/
[24] As expressed by companies at a hearing in the EU Parliament on 18 July 2023, https://www.europarl.europa.eu/committees/en/how-to-make-sure-that-pesticide-manufact/product-details/20230704CHE11961 accessed 2023-09-13
[25] In accordance with Regulation 283/2013 Annex point 1.2