Pesticide Action Network Europe

MISSED & DISMISSED

PESTICIDE REGULATORS IGNORE THE LEGAL OBLIGATION TO USE INDEPENDENT SCIENCE FOR DERIVING SAFE EXPOSURE LEVELS

générations FUTURES

I. Summary

All literature, including *independent* studies (i.e. science financially independent of private interests), now need to be taken into account when the EU Commission and EU member states (MS) discuss whether to approve a pesticide for sale. This is what politicians decided in the 2009 pesticide Regulation 1107/2009.

PAN Europe analysed a sample of seven of these new pesticide dossiers (risk assessments, RA) to see if the pesticide industry and governments are living up to this provision. We discovered that only 23% (99) of 434 important toxicity studies from academia were identified by industry in these seven RA. Further, not one of those 99 studies was seen as relevant and reliable enough to be used for decision-making, generally because they were not performed according to OECD-test protocols (including Good Laboratories Practice, GLP). A guideline of Food Authority EFSA showed industry the way for dismissing these independent studies. Yet independent scientists regularly find risks likely because of the more sensitive methods of detection used. For the seven pesticides in the sample of PAN Europe, we found several studies falsifying the alleged RA safe doses (based on a chronic exposure 'key study') from 2 to over a 1.500 times.

The MS overseeing the dossiers allowed industry to disqualify the independent studies without any logic, and didn't demand a revised assessment before authorizing a pesticide's use, as politicians envisioned their doing. MS mostly did not even ensure industry's mandatory search for independent studies were performed – Spain even claimed, without rationale, that there is no such mandate. Sweden did call for some independent studies to be considered in one particular risk. PAN Europe is very concerned about the way democratic EU decisions turn into a "dead letter" when implemented at the Commission and MS. The EU institutions and stakeholders must discuss this.

II. Introduction

For decades, pesticide regulation was based on industry-sponsored toxicity studies. The work of independent scientists generally was not collected or taken into account. It therefore took a long time before the new insights of independent studies were accepted by the Brussels community; despite a long-standing requirement in pesticide Regulations to base any authorization decision on current scientific and technological knowledge.

A chemical RA to find a safe exposure level of an agent begins with a literature review. EU agencies as elsewhere adhere to a rule of the Organization for Economic Cooperation and Development (OECD) called Mutual Acceptance of Data (MAD).[1] MAD requires RAs done to allow agents onto market to use the OECDs 'Test Guideline' (TG) – standardized toxicity test methods (for cancer, neurotoxicity, etc., and a generic one called Good Laboratory Practices (GLP). These test methods originated in the organic chemicals industry[2], and appeared at OECD immediately followed the GLP anti-fraud regulation appeared in the USA, after a whistle-blower revealed blatant and universal fraud at industry laboratories. In five years, TG-GLP was required in pre-market RAs globally. Critically, the TG methods are rejected by independent scientists as restrictive of their freedom to investigate, so a *de facto* but total barrier to the latter's studies use in RA exists. Thus citizens are in the absurd position of having their risks determined with very insensitive methods by the party whose every interest is for their agent to be found safe enough to market.

Major insensitivities of the TGs test methods include:

- their chronic doses are actually quasi-poisonous, so *do not reflect what happens at realistic doses;*
- few exposures during vulnerable development;
- no time for chronic disease to develop is allowed before the animals are sacrificed;
- unrealistic controls can cause toxicity to disappear.[3]

[1] www.oecd.org/env/ehs/mutualacceptanceofdatamad.htm

[2] *Buonsante V et al. 'Risk assessment's insensitive toxicity testing may cause it to fail.' accepted Jul 2014 Environ Res.*

[3] *Haseman JK: Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. Environ. Health Perspect 1984, 58:385–392.*
Hardisty JF: Factors influencing laboratory animal spontaneous tumor profiles. Toxicol Pathol 1985, 13:95–104.
Myers JP, et al.: Why public health agencies cannot depend on good laboratory practices as a criterion for selecting data: the case of bisphenol A. Environ. Health Perspect 2009, 117:309–315.

New Regulation 1107/2009

Recently however, EU lawmakers have begun requiring industry to identify and evaluate "all available data" when doing a pre-market RA—e.g. in the REACh law regulating chemicals, and for biocides and pesticides. Pesticide Regulation 1107/2009 for example requires manufacturers to make a review of the "scientific peer-reviewed open literature". [4]

For the 30 pesticides (active substances) whose 10-year authorizations are the first to expire after this mandate entered into force[5], manufacturers now must review *all* available data. The company or industry (the Applicant/Notifier) proposing an approval must submit this literature review to the EU country volunteering to evaluate the active substance (Rapporteur Member State, RMS), together with all other required safety tests and studies. The *RMS must approve the completeness of this dossier[6], including the literature review*; part of the process of composing a summary dossier, in a dialogue with the applicant. This Revised Assessment Report (RAR for those pesticides being re-authorized) covers all RA issues including assessing every exposure scenario, resulting in several volumes, sometimes over 1000 pages. After RMS is satisfied APPL has supplied all the data needed to assess risk, the RAR is sent to European Food Safety Authority (EFSA) and the other Member States for comments. EFSA then must take public input on the RAR. With all input, EFSA performs a peer-review on the RAR, the basis (a recommendation) for the final authorization (or not) via a majority vote (qualified) by the Standing Committee, a political body of civil servants selected by the (generally agricultural) ministries of MS; using a proposal prepared by DG SANCO (unit E3) from EFSA's recommendation and political demands from Member States.

[4] *Regulation 1107/2009, Art.8.5: "Dossiers, The summary dossier shall include the following: Scientific peer-reviewed open literature, as determined by the Authority, on the active substance and its relevant metabolites dealing with side-effects on health, the environment and non-target species and published within the last 10 years before the date of submission of the dossier shall be added by the applicant to the dossier."*

[5] *COMMISSION REGULATION (EU) No 1141/2010 of 7 December 2010 laying down the procedure for the renewal of the inclusion of a second group of active substances in Annex I to Council Directive 91/414/EEC and establishing the list of those substances*

[6] *Regulation 1107/2009, Art.9.3: "Where the dossiers submitted with the application contain all the elements provided for in Article 8, the rapporteur Member State shall notify the applicant, the other Member States, the Commission and the Authority of the admissibility of the application and start assessing the active substance."*

Rationale for our investigation

Because the crucial step in the pesticide authorization decision ***is the gestation of the RAR in a dialogue between industry and the regulatory agency of an MS*** *– others are only allowed to see the RAR after its draft conclusion is reached–* we were very interested to see how these secret parties carried out the critical new mandate, to assess "all available data".

This report thus audits whether the released RAR contains any of what we think are the most relevant published toxicity findings of financially independent academia. All findings must be reported, but we focus on *in vivo*, chronic exposure studies, especially low doses and for endpoints often unstudied in RA. This is both practical (our limited auditing resources) and useful, as these studies would be key studies to set the safe dose on. Of course the risk of other exposures are also considered in a RA, and because academics very often study ecosystem toxicity, we also audited if that literature was found by a RAR.

When a RAR did identify a toxicity study we considered significant, it was especially important to see how its findings were treated, given the above failures of RA. After the new 2009 mandate, EFSA issued a controversial guideline on how to do such reviews of scientific peer-reviewed literature[7]. EFSA was caught between the new mandate and its usual RA methods, where academic studies play no role whatsoever. PAN Europe was concerned[8] that this Guidance would frustrate the EU's intention in the Regulation, allowing the effective ban on independent toxicity studies from RAR to continue despite the new mandate. The central element of the Guidance is a 'Klimisch score', named after a 1997 published paper by three BASF-employees[9], which gives industry-sponsored (and TG-GLP compliant) studies the top reliability score, while independent academic studies – which do not use TG-GLP – get a Klimisch reliability score of 2, 3 or 4.

Ideally, "reliable" toxicity data comes from a test that is accurate (both sensitive and specific) and precise (reproducible). **But when health is the goal** (e.g. in RA), enhancing ***sensitivity* – the ability to detect toxicity** (find true positives) – **must override the ability to be *specific*** (avoid false positives). RA's toxicity tests promote the reverse.

Using the Klimisch score as the only means of assessing data reliability means that evaluators do not need to evaluate studies, but only check if a study used the OECD TG protocols in a GLP-certified lab (and the study also GLP-certified). This means that far more scientifically advanced studies are bureaucratically dismissed. A critical, or systematic review of "all available literature"[10] is bypassed by the Klimisch check mark…and it is universally in use in RA. We thus also audited EFSA's own test of their Guidance –by the Austrian agency AGES, commissioned by EFSA to test the ability of EFSA's Guidance in finding "all available literature".

Finally we wanted to show the impact of ignoring "all available data". A RA is largely driven by the lowest of the "no effect" doses (NOAEL, 'no-observable adverse effect level'; or at least a LOAEL, lowest observed adverse effect level) in the chronic exposure toxicity tests, leading to the Acceptable Daily intake (ADI, after some safety factors for unknowns are applied to the NOAEL). The chronic ADI largely drives if and how much of an agent is used. So we highlighted when an independent toxicity finding (whether the RAR found it or not) is below the pesticide's key study chronic NOAEL. In this way we demonstrate the *consequence* of ignoring the independent literature generated by academic scientists.

[7] *EFSA guideline, 2011, www.efsa.europa.eu/en/efsajournal/pub/2092.htm*

[8] *See: www.pan-europe.info/News/PR/101020.html*

[9] *H.-J. Klimisch, M. Andreae, U. Tillmann, A Systematic Approach For Evaluating the Quality of Experimental Toxicological & Ecotoxicological Data, Regulat Toxicol & Pharmacol 25, 1–5 (1997).*

[10] *Investigated in: http://policyfromscience.com/the-future-of-evidence-in-chemicals-policy/ and other pages at that site.*

III. Report methodology

- Random selection: Driven simply by the availability of RAR's (finalised by the RMS then published by EU Food Safety Authority EFSA[11], found on their webpage on pesticide Assessment reports[12]), our only selection bias was to choose pesticides well-studied by academia, allowing us to perform our comparison. We guessed a minimum of 8 published academic studies was needed, so of the ~30 pesticides being re-authorized we ended up auditing seven, on the new mandate to find 'all available data'.

- Peer-reviewed publications from academia (required to be reviewed by the Regulation) are most reliably found in PubMed (run by USA federal National Institutes of Health), which indexes the published studies of essentially all journals in the life sciences (often only the abstract is available). PubMed indexes more journals relevant to toxicity than other database; critically, this is where the high-quality (peer reviewed, published, and still mostly financially-independent) studies are.

- To check compliance with the new mandate, we performed a simple search of PubMed: "<pesticide common name> toxicity". Abstracts use chiefly the common name of a chemical (see also our below discussion), while the later is a specific PubMed keyword. Tested against PubMed searches without the qualifier 'toxicity', such a search term typically captures circa 95% of published toxicity studies. While it misses the grey literature (including original toxicity studies by industry and government labs), it is obviously more important to capture the studies of academic scientists.

[11] http://dar.efsa.europa.eu/dar-web/consultation

[12] http://dar.efsa.europa.eu/dar-web/provision

- After eliminating the most obviously irrelevant, we saved the remaining studies (by publication date) in a publically available collection (whose numbered abstracts are used to identify studies in our tables).

- Next, we read and scribbled marginalia of a pesticide's resulting abstracts, to identify the most important academic findings on its toxicity e.g. potency, synergy, or rarely tested endpoints as immune, endocrine or developmental neurotoxicity. If needed to clarify the findings, we read the entire article.

- Critique of the RARs: For each of the seven selected RARs we searched the pdfs of its main Volume 1, its list of studies (Vol. 2), and the human & ecologic risks (RAR Annexes B6 & B9) for the first author name of each academic study we identified above, as the RARs refer to any study by first author name.

- In the annex, see a table for each RAR into which we placed the results of the above analysis, sorted by each of most major toxic endpoints; containing:

    1. The number of studies we found versus those found by the manufacturer.
    2. Whether the studies found by the manufacturer were utilized in the RA or dismissed; and if the latter, on what grounds.
    3. We critique the RMS's assessment of the industry literature review.
    4. Whether our lowest dose in vivo and chronic exposure toxicity findings were utilized in the crucial RA task of finding a lifetime safe exposure dose, the ADI.

IV. Results

All results are summarized in our Summary Table, while detailed results for each pesticide are in an Annex Table for each pesticide.

1. Quality of industry's literature search

The seven varied a lot in the extent of their literature search; from Thiabendazole whose applicant and RMS seemed unaware of the mandate and so mentioned not a single academic study; to four (amitrole, l-cyhalothrin; esfenvarelate, fenhexamid) whose compliance effort was minimal; to two with large published literatures (2,4-D and glyphosate), whose RARs did describe the literature searches made.

Overall, RMS did not put much energy in evaluating the literature reviews of applicants. For example, Germany stated that it only checked a limited number of papers on the herbicide 2,4-D. UK on Fenhexamid simply agreed with the applicant without commenting. Spain on Thiabendazole even discouraged the literature review entirely, saying it is not mandatory.

No-one checks the RMS's compliance decision on the manufacturer's dossier, and the Commission (DG SANCO) has *always* accepted the RMS's literature search compliance check, so the public remains in the dark about the real risks of a pesticide it is exposed to. Based on the public consultation, EFSA might take a look at some independent studies in their peer-review.

2. Quality of the RMS assessment

Not finding many of academia's studies *is not the only cause of the failure of pesticide RA's*. As discussed, RARs invariably recommend use authorization be granted based on the toxicity studies of the party whose revenue depends entirely on their product being found safe enough to use. While evaluation of the quality of studies is mandatory, it is just as mandatory to justify with scientific objectivity the basis for accepting or rejecting data. RAs, including RARs fail to do this.

For amitrole. RMS France issued a RAR completeness check on a non-existing literature review! Only Sweden and Holland commented that l-cyhalothrin and thiabendazole (respectively) literature reviews were inadequate, for a specific endpoint anyway; and cited relevant studies, urging it be considered, at least in the future. Yet several times (see Summary table; especially for l-cyhalothrin), the exact data–endocrine disruption, developmental neurotoxicity, etc.–that these few RMS ask for is waiting for them in databases such as PubMed…if only they and industry would see that the independent literature it is reliable!

Not one of the 23% of independent studies (99 of the total of 434 studies we found) was used by a RAR to determine the safe chronic exposure dose, the ADI. Nor did they much influence the use restrictions for ecotoxicity (although we did not check the RAR ecotoxicity study evaluations rigorously, this is quite obvious in most RARs).

3. Relevance and reliability evaluation of studies

As stated, none of the 434 independent studies (or even found) we identified were considered reliable enough for EU decision-making; nor were any of the several thousands other independent studies identified by applicants (e.g. the applicant of Glyphosate stated they considered 2000 studies) selected as a reliable key study. Neither did any of the MS ever disagree with the applicant.

4. Falsifying the RAR's Chronic 'Safe Dose'

Because we had to scan all of a pesticide's literature in PubMed to see which ones an Applicant missed, we saw findings of low dose toxicity. And because we had to select pesticides with a large independent literature, we noticed what we have become accustomed to in all RA: independent academics had falsified the key chronic toxicity study (used to set the safe dose) of each one. The one exception *proves the rule*: fenhexamid had no independent *in vivo* chronic studies *at all, despite* wide & long term use (perhaps the manufacturer does not allow academics access to it?).
So for each RAR where our Annex table lists the key finding(s) of independent studies, we highlighted in yellow any low dose falsification of a claimed NOAEL or other important RAR safety claim (sorted by endpoint: neurotoxicity, cancer, etc.). However, independent academics have overall not quite replicated the study designs of RA TG-GLP tests (chronic oral dosing of rodents). Of the six falsifications we find, one used injections instead of oral dosing (glyphosate), and two were in non-mammals (but still vertebrates) (amitrole and glyphosate). See also our Summary table for brief descriptions of these especially important studies. In general we highlight *in vivo* chronic exposure studies; but we also highlight any low dose toxicity finding, including for ecotoxicity.

Note more falsifications of the RARs simply await investigation by independent academics.

THE FALSIFICATION IN VERTEBRATE, USUALLY MAMMAL OF CLAIMED SAFE. LIFETIME-EXPOSURE DOSES

5. Critiquing EFSA's Test of their Guidance On Finding Literature (the AGES Report)

After publishing its Guidance on how to fulfil the new mandate to find "All Available Data", EFSA contracted with the Austrian federal Institute for Plant Protection Products (AGES) to test literature search methods for three pesticides.[13] The Guidance is thorough in many respects, and applicants could take advantage of it to make the broadest possible searches (as to search subject and data sources), making full use of EFSA's Guidance and AGES's recommendations.

[13] www.efsa.europa.eu/en/supporting/pub/511e.htm

Yet, despite the availability of AGES's EFSA Guidance-based search recommendations, applicants found (as already mentioned) just 23% of the toxicity studies we deemed relevant (we had not the resources to test the AGES/EFSA search method for the subjects of food residues, environmental fate, resistance, etc.). We specifically tested AGES' method for just one of the three pesticides they searched, Metalaxyl-M: *not one of the 18 relevant toxicity studies (many in the gray literature) that AGES found in its more comprehensive search for this fungicide is one of the six toxicity studies we found in a PubMed search*. So, while the AGES method will find some studies we do not find; it will not find others, especially critical ones from independent academia.

The AGES report acknowledges that searching databases without a pesticide's trade name did not reduce the number of studies found (its p. 46). PAN-Europe's methodology uses only the common chemical name of a pesticide. We find that PubMed misses a small proportion (5-10%) of published studies whose abstract used a trade name, the pesticide's full chemical name, or its unique CAS #, as the AGES searches did; because academics are attentive to details, to refer to a common chemical name.

AGES discusses it at length, but we do not think the '*relevance'* of a found study is controversial—any study's aspect to a risk assessment is relevant. In contrast, as we discussed, we do feel that assessment of study "*reliability*" is controversial. Both EFSA and AGES (by recommending industry use the ToxRTool to evaluate study quality) rely on Klimisch to dismiss without analysis any study not performed by the party with a tremendous financial incentive to find safety. As discussed, Klimisch relies on bad logic—a TG/GLP study is *de facto* of high quality i.e. reliable, regardless of its actual methodology.

In sum, AGES used the EFSA Guidance on finding peer-reviewed literature in a very narrow sense. Though thorough in finding sources of information, AGES prioritized the Klimisch criteria instead of a critical / systematic assessment of all available literature. The conclusion of the AGES study therefore is that independent studies are not reliable (do not follow the OECD TG-GLP protocols). This is not in any way scientific.

V. Discussion and recommendations

We wondered since 2009 what would happen when the first mandatory reviews of independent literature…and our worst fears are confirmed in our analyses. Industry found only 23% of important published studies. Worse, they decided to qualify none of them as reliable enough to use in determining risk. As always, they took advantage of the EFSA Guidance to use Klimisch to dismiss without analysis these and thousands more studies (some of which would be useful, though we did not look at them) on these seven pesticides alone. This means industry and *regulators have not even read these studies and so cannot assess their quality…but do so anyway*. Remarkably, none of the RMS protested against the simplified assessment (only Sweden and Holland urged limited consideration of academia's literature). Nor has DG SANCO filled its role in guarding against these failures by industry and MS.

This report finds *exactly* what two previous NGO audits found in RAs to Register chemicals under the EU's general chemicals REACh law.[14] Evidently it is pointless for the EU institutions to require that "all available data" be assessed in RAs, if *they do not also ensure that the most reliable science is allowed into RAs*.

This is our key message: academia's toxicity studies produce far more reliable data (being more sensitive--very few false negatives), yet the insensitive tests are still determining the outcome of RAs, as evidenced by their complete dismissal, here and in REACh.

[14] *EEB (European Environmental Bureau), ClientEarth. 2012. Identifying the bottlenecks in REACh implementation-the role of EChA in REACh's failing implementation. [Available: www.eeb.org/EEB/?LinkServID=53B19853-5056-B741-DB6B33B4D1318340 .*

ClientEarth. 2013. REACH registration and endocrine disrupting chemicals. Available: www.clientearth.org/reports/reach-registration-and-endocrine-disrupting-chemicals.pdf

Critical Analysis of RA

A key hypocrisy is at work in pre-market RAs globally. To dismiss an independent study from academia, an RA first properly summarizes its methods and findings. But *defined criteria to analyse study quality* are not then deployed – rather, one or more weaknesses of the study are noted as the reason to dismiss its use in the RA. Any study has flaws, but in RA there is never any evaluation of the *relative* merits of TG and non-TG studies competing to describe potency or a toxicity endpoint, so no one can evaluate whose data is more reliable. Instead, it is ***assumed*** that TG-GLP test methods of the applicant produce the most reliable data. Their positive attributes (such as standardization and careful dose ranging (at high doses anyway) are cited as markers of reliability. Their weaknesses may or may not be noted, but these are never, in the hundreds of pre-market RA we have read, cast as making the study unreliable. Instead, Klimisch ranking is used to easily execute their judgement, instead of using critical criteria. This is why academia can so easily falsify these claimed safe doses with findings of greater toxicity. Even SANCO Commissioner Dalli testified to Parliament that *"…it is correct that GLP does not evaluate the scientific quality and reliability of a study…"* (7-4-2011).

That is not to say that the lowest dose finding should automatically be the basis for the safe dose. Even the best-studied chemicals (here, 2,4-D and glyphosate) have *more unknowns than knowns*. Each experiment is a small part of the picture, and every study has flaws. So while a finding of toxicity at lower dose does until further notice falsify a claimed no-effect dose, further tests are needed. One issue is the species used. Our review found low dose chronic toxicity across a broad range of species, raising question as to their relevance to humans. Segments of the human genome is conserved across life taxa[15], so findings in vertebrates, or even in invertebrates, are relevant to humans (and others'!) risk. E.g. for amitrole, low dose thyroid toxicity in fish is relevant to humans, but demands confirmation in mammals—here we show all corroborating evidence of amitrole thyroid toxicity we could find.

"Falsifying' a result is not controversial; it happens to many, many science studies. **But it does mean that the falsified result cannot be relied on in any way**. In RA, a finding of toxicity at a dose lower than a RA's claimed safe dose ought to trigger further toxicity studies, until the safe dose becomes clear. Instead, unelaborated and utterly unsupportable claims about data reliability are made and approval is granted. This farce must end.

This ultimate argument is driven by study design. To date, industry and regulators argue that reproducibility and specificity (reducing false positives) are the key needs of a toxicity test, but *the very goal of RA (protecting health)* indicates that sensitivity to detect effects is the over-riding need of a toxicity test. Developmental vulnerability, allowing disease to develop (instead of destroying the evidence), testing realistic low chronic doses instead of the end poisoning (which returns an alleged "NOAEL"), proper use of control animals, and issues surrounding dose route & metabolism/excretion are the major problems making RA insensitive…but agencies wont discuss them!

[15] http://en.wikipedia.org/wiki/Comparative_genomics

There is a wide gap between the scientific and regulatory worlds. Academics are interested in what happens after exposure. Using instead the less sensitive toxicity findings of industry (via the Klimisch evaluation), tens of thousands of peer-reviewed very reliable toxicity findings from many thousands of independent scientists are *effectively thrown into the trash by RA*. This, despite decades of the EU law saying that current scientific and technological knowledge must be the basis of any EU decision taking.

New insights in science are taken very late on board in EU decisions, if at all. Even today pesticides are not tested for endocrine disruption, despite such effects being known for over 20 years. EU politicians and the EU Parliament tried to bridge this gap with the clear requirement in the pesticide legislation to review independent literature as a part of the dossier. EFSA was the first to weaken the requirement, presenting an opinion saying that the assessment can be narrowed down to a few simple criteria based on Klimisch, elevating insensitive TG-GLP protocol studies.

Accordingly, we recommend:

- Revise the EFSA Guidance because there is no critical review/ 'weight of the evidence' review of the independent studies (rather, just the Klimisch procedure);

- For re-authorizations, independent academics should perform the literature review before the applicant submits the RAR to RMS approval. All studies, including sensitive ones, would undergo a 'weight of the evidence' critical review (i.e. using pre-defined data reliability criteria) by RMS, who would commission academics to fill significant data gaps, and identify a NOAEL &/or LOAEL for each endpoint, and base the ADI and other 'safe dose' determinations on this critical review;

- The literature review in fact should be done before industry starts doing the required safety testing. Based on the literature review a more complete hazard profile of the substance might be found in academic research. For each of the identified hazards, industry has to test the substance at relevant doses of exposure, based on the current data requirements, and employ additional tests for identified hazards

- For new pesticide authorizations (with little study by academia yet), the control of toxicity tests must be wrested away from industry and given to academia.

- Revamp the RAR completeness check at the national level and require thorough control of completeness at the European level, including the literature review;

- Begin dialogues between regulators and academic & other scientists, on the determinants of reliable toxicity data. PAN-E is determined to change agencies' "TG-GLP" RA method, and we hope other stakeholders are too (Germany's federal risk assessment agency BfR has asked the pesticide Standing Committee to discuss such controversy, prompted by an NGO report from scientists documenting such failures in the RA of glyphosate[16]). Primarily, this requires extended dialogue on the determinants of a reliable toxicity data between these agencies and leading academics that are finding toxicity at low doses (including pesticides). The natural forum for this dialogue is the OECD's 'WNT' committee, as it not only creates and modifies toxicity test methods for use in RA globally, but also its members are leading regulators in those national agencies, such as EFSA and DG SANCO.

[16] Roundup and birth defects - Is the public being kept in the dark? At: www.earthopensource.org/files/pdfs/Roundup-and-birth-defects/RoundupandBirthDefectsv5.pdf

PAN-EU 'Missed & Dismissed', Summary Table

Pesticide	Literature Review by applicant?	Literature Review evaluated by RMS?	% our studies found)	Used in ADI?	Chronic NOAEL (RAR's), for ADI	In-vivo academia LOAELs < RAR NOAEL	Low Dose In-vitro academia findings	Remarks
1. 2,4-D	Yes, 147 pages; of total of 12.000 references, 500 were assessed for potential relevance; 177 are mentioned in the review, abstracts included.	"Member State reviewed … only for a limited number of these references…in certain cases, there was missing information on the notifier's report from the provided literature. … it wasn't possible to review all original reports / publ's". RMS supports APPL in dismissing links with cancer, saying 'inconclusive' and dismissed cancer epidemiology studies due to lack of statistical precision, low sample size or exposure misclassification. The non-Hodgkin Lymphoma link is called inconsistent and inconclusive. Industry's & RMS sum: not a carcinogen.	26% (n=27)	0%	NOAEL 5 mg/kg d- LOAEL 62,5 mg/kg d-	#8: 2.5 mg/kg d- (behavior, oral, rat). #38: 3,3 mg/kg (genotox, oral, mouse). #43 10 mg/kg d- (EDC, oral, ewes). Dalgard '93b: incr. thyroid wt. 1.6 mg/kg d- (90 d, oral, dog, TG-GLP).	#25 (Bharadwaj '05) 0,01 mg/L (altered gene expression).	In sum, reliable (independent, consistent) evidence of changed level of neurotransmitters, changed gene expression, and cancer (NHL), much at low doses.
2. Amitrole	No. A single academic result mentioned on thyroid disruption in fish. Relevance defined; Klimisch for reliability	No sign of serious RMS France evaluation & they agree on evaluation criteria. They claim only 1 new academic study since last authorized!	0% (n=27)	0%	NOAEL 0,1 mg/kg d- LOAEL 0,35 mg/ d- (from US-EPA)	Li et al. '09: 10 ng/L fish thyroid, & genes altered. Assuming inhaltn. exposure, this is ~ 1 μg/kg d- Johnson'81 (2 yr. rat oral: follicular thyroid cancer at 0.25 mg/kg d- (no NOAEL). Fregley '68: decrease in iodide at 0.5 mg/kg d- (NOAEL: 0.1 mg/kg d-).	Furukawa, 2010: 20 μM DNA damage.	USA disagrees, says probable human carcinogen-liver, thyroid in rodents, consistent w/ Li '09. Johnson '81 & Fregley '68; all low dose risk indications. EU: R2; suspected of damage to unborn child.
3. Fenhexamid	Yes, search revealed 616; just 3 called relevant & discussed.	No sign of serious evaluation (UK); they simply agree with the APPL	11% (n=9)	0%	NOAEL 19 mg/kg d- LOAEL 137 mg/kg d-	(Data gap from academia).	#3: anti-androgen 10 nM incr. miR-21, breast cancer protein	Unusual serious data gap from academia for a long marketed chemical, incl. no in vivo studies.
4. L-cyhalothrin	Yes, L-cyhalo ~ 3 fold more potent than cyhalo mix. 'Some' endocrine disruption (6 in vitro) & immunotox (2 in vitro); 4 aquatic tox.	Sweden: no developmental /reprotox evidence & DNT uncertainties, so Sweden advises increase the uncertainty factor and re-evaluate once EU decides on EDC criteria.	36% (n=33)	0%	NOAEL 0.5 mg/kg; LOAEL 1/3.5 mg/kg d- (an extra 3-fold safety factor to ADI, for lack of DNT).	#3: 0.2 mg/kg d-, reprotox, mice. #'s 8, 9 & 13: 0.1 mg/kg d- NOAEL; 1 mg/kg d- LOAEL; dev. neurotox.	#62: 1 ng/L genotox.	Syngenta challenges EU classification I for ED based on in vivo Ahktar, '96. Sweden agrees as formulation not active agent, was tested; yet would not dismiss the findings. Even industry notes Wistar rats for DNT are less sensitive.
5. Thiabendazole	No evidence of a literature review.	RMS Spain feels the review is not obligatory; Co-RMS NL disagrees says a quick search shows studies with estrogenic properties	2% (n=65)	0%	NOAEL 10 mg/kg d- LOAEL 40 mg/kg d-	#4 : 0.7 ug/L (immune, amphibian). #61: teratogen, oral mice: 26.4 mg/kg d- (ED_1)	#3: 0.5 μg/ml aneuploidy.	
6. Esfenvalerate	Yes, but no information provided.	No serious evaluation; RMS UK concludes literature search acceptable as to databases searched, search terms Not relevant tox/ecotox section.	0,7% (n ~130)	0%	NOAEL 1.75 mg/kg d- LOAEL 1.9 mg/kg d-	#54: 0.45 mg/kg d- neurotox, rat. #13: 0.19 mg/kg d- semen, mic; FEN read-across #23:<6 μg/L fish genes, FEN read-across. #21: 8–14x aq. tox synergy w/ prochloraz	#8: 5 uM reprotox #131 0.2 μM genotox (both Fen read-across)	Fenvalerate is mix of isomers of which esfenvalerate is 23%, the basis to 'read-across', i.e. to use toxicity studies of the former.
7. Glyphosate	Yes, a review of 860 pages, 2000 studies was assessed and 1000 were assigned a classification (Klimisch and other) yet many were never discussed. About half the ones we thought important were not found in their large review.	RMS agrees with industry on all aspects of literature search and reliability evaluation.	52% (n=146) (Just 31% discussed).	0%	NOAEL ~100 mg/kg d- LOAEL ~300 mg/kg d- ADI selected is mid-point of industry & RMS, proposals yet less conservative than previous ADI.	LOAEL ~10 mg/kg d- Monsanto study (rabbit)! #93: Teratogen to chickens & frogs, 1:5000 dilution of product of glyph alone. #41: ~0.2 mg/kg d-: anti-oxidant enzymes (rats, 30-90 d exposure). #113: 1:250 dilution LD50, neurotox & synergism w/ 2 common pesticides; detailed mechanism elucidated.	Thongprakaisang et al.'13, 10(-12)M (pptr) E2 mediatd prolifertn @ < popultn. burden. #122: genotoxicity 1/10,000 dilution: population body level. #36, #125: Aq. tox 0.05 mg/L & 2 ppb.	The Monsanto rabbit studies showed a non-monotonous dose-response, most potent at 10 mg/kg. Thus no NOAEL was established, yet applicant & RMS Germany simply dismissed the study.
AVERAGE			23% (n=434)	0%				

Legend

APPL	Applicant: the manufacturer/s of the pesticide sold/imported in the EU.
1 → *n*:	Number of the saved PubMed abstract (occasionally a short cite, if found outside our PubMed search.
Short cite:	(e.g. " Smith '09 ") For a few studies not found via PubMed, below each table is list of their references.
A → Q	The 2,4-D table alone cites a few from an additional collection denoted A to Q; also referenced list.
(*n*):	Brackets around a single study number denote we did not double count (as to num. applicant found') a study multiple-listed for different effects (does not apply if brackets are around more than one study number). (the same applies to a handful of studies that appeared after a DAR, which we wanted to discuss anyway).
Red:	Simply for emphasis.
Blue	A notable low dose finding.

Note on reading the tables: Although no guiding lines are used, related information is aligned 'top to bottom' across columns, accounting for visual appearance of blocks of text.

2,4-D (herbicide)

RMS Germany confirms 2,4-D's previous lifetime safe exposure level (authorised 01-10-2002), based on a published industry study (JM Charles et al. 1996. Fund. Appl. Toxicol. 33:166-72) where the LOAELs were between 62.5 and 150 mg/kg a day, (gender dependant); NOAEL **5 mg/kg d-**, and 100-fold safety factor the ADI is 0.05 mg/kg d-.

	Peer-reviewed toxicity studies found by PAN EU	Found by APPL?	If 'Yes, How Was It Considered? What else was Considered?	RMS Comment & action taken on literature review	PAN Europe comments
Summary	27 studies with important adverse effects of 2,4-D (many at low dose).	26% (7/27);	APPL dismissed all seven (100%). General reasons given: - in-vitro studies on genotoxicity are questionable - in-vivo studies on genotoxicity not convincing - reprotoxicity only at high doses - some evidence on endocrine disruption but not substantial and weight of the evidence effects is lacking - neurologic effects only indirect immune effects not shown because of poor study design.	No action taken on the failure to take into account much of the open literature.	Our simple search term search returned 300+ published 2,4-D toxicity findings, of which we selected 26 as likely to affect the RAR's result. Despite industry's extensive literature search, they only found 27% of our 26 important studies; so no doubt other published toxicity findings were ignored.
Organ/System					
Reproductive Toxicity			In general: "…generally unaffected … at doses up to and including 20 mg/kg b.w./day "[This study's effect] not replicated in 1-gen. test at any dose up to the renal clearance rate" Weaker at lower doses. Small groups.		Hypocritically, the RAR does not similarly cite "weight of the evidence" method for assessing cancer risks (below)
	8: Lactation decreased and hormones altered at just 2.5 mg/kg (single dose inj., or daily in food).	Yes			Lactation is not measured in the 1-gen test--how can APPL claim above? 'Significant' finding takes small study into account. D/R indicates causation. Other criticisms don't affect this result.
	A: High dose fetotoxicity	No	"…[teratogenicity] at [other study] top dose of 75 mg/kg b.w./day….not teratogenic."		(A) agrees with APPL's summary—so why called non-teratogenic?
Endocrine Disruption	22: *In vitro* low dose androgenic	Yes	"Overall the weight of evidence indicates a lack of effect of 2,4-D on steroid hormone mediated systems."	In general: "some in vivo studies provide evidence for endocrine effects…especially in thyroid hormone system. Overall, although there is no substantial evidence of [various ED] effects in the available F1-extended one generation reproduction study in rats …further discussion of this issue is proposed in a meeting of…experts at EU level."	No reason given why this *in vitro* study disregarded.
	43: Metabolic/ reproductive endocrine toxic effects	Yes	"This study gives limited evidence for lack of endocrine-disruptive activity in ruminants."		10 mg/kg for 36 d in sheep, oral dose. … so it gives clear evidence of ED.
	Dalgard '93b in RAR: incr. thyroid wt. at 1.6 mg/kg d- (90 d oral, dog, TG-GLP)	Yes/No	Dismissed simply for using dog, a mammal.	EFSA also claimed (in previous DAR) this effect is not adverse.	Though summarizing it, the RAR never mentions this low dose effect! What evidence by EFSA previously that this statistically significant change is not adverse?
Genotoxicity	I*n vitro:* (15, 25, 26, 30, 32, 34, 47, E, G, H, M, Q)	Mostly No	RAR says 2,4-D is largely not genotoxic; except: "…gene mutations *in vitro* is questionable, whereas the *in vivo* potential has not been adequately investigated. As a first step, an *in vitro* mouse lymphoma assay is required."		*in vitro* genotoxicity as low 100 ug/L and 0.1 nM;
	I*n vivo:* (16, 34, 38)	Mostly No			*in vivo* genotoxicity 3.3 mg/kg d- (38) was missed.
Cancer	(See PAN-EU comment, far right).	Yes	"…There are no [experimental] indications of carcinogenicity."	"…Overall, the RMS concludes … there is no substantial [epidemiologic cancer] evidence that 2,4-D may exhibit toxicological properties other than those concluded already based on the [experimental] toxicity studies conducted with the technical active substance..."	Contrary to RMS' claim, there are dozens of published associations of 2,4-D with several cancers, including blood cancer. No one can distinguish if it is 2,-4-D or its known-carcinogenic dioxin contaminants that are carcinogenic; so we did not audit the APPLs inclusion of these. We note, (http://www.abc.net.au/news/2013-07-22/four-corners-dangerous-dioxins/4833848), that 2,4-D produced in China, exported (unknown if 'substantial equivalence' regulations prevent its import into EU) today has very high levels of dioxin despite long-standing claims by the industry that manufacturing changes drastically reduced dioxin levels.

	Peer-reviewed toxicity studies found by PAN EU	Found by APPL?	If 'Yes, How Was It Considered? What else was Considered?	RMS Comment & action taken on literature review	PAN Europe comments
Synergistic Toxicity	Various *in vitro* studies find 2,4-D toxicity increasing in presence of other agents vs. tested alone (3, 11, 18, 19/20, 29, 35, 48). *In vivo:* 33; Rosa et al. '05; 23.	No (29, yes)	(29): "Not relevant for risk assessment, no potentiation of oxidative DNA damage by 2,4-D."		(29): Untrue—see above genotox findings. No finding should be so baselessly dismissed. Note EFSA is only now beginning to perform cumulative risk assessments. Immune suppression (see below).
Neurologic Toxicity			In general: "…no neurotoxic potential … was identified based on the above findings."		
	13: Behaviour toxicity at 15 mg/kg d-	Yes	Effects might be due to maternal toxicity from high doses (given the excretion rates).		*APPL deemed about the same dose acceptable enough to support the safe dose!* — so why is it suddenly "too poisonous (high dose) to represent chronic toxicity"?
	O, Rosso et al. 2000.	No, No			*in vivo; in vitro* (both high dose)
Immune Toxicity	I or 40 (same paper, but 40 is Emel'baeva et al. '99 or '00).	Yes	"The significant issues with the design of this study preclude consideration of the results for human health risk assessment."		Dismissal relies on only one dose, but PubMed abstract says 2 & 20 mg/kg d- were tested.
	33; (de la Rosa et al. 2005); 23.	Yes, No No	"This study was conducted at dose levels that exceed the threshold for saturating renal clearance of 2,4-D. As a result, the internal dose would have exceeded the range of linear toxicokinetics. Intraperitoneal injection is not a relevant route of administration for evaluation of human health risk assessment."		Doses were 50 - 200 mg/kg d- (7 days)—the same as many studies the RAR did accept. If injection is not realistic, how can internal dose be relied on to dismiss the study? Consistently at this lab (all three studies) 2,4-D synergistically or alone caused immuno-suppression.
Metabolic Dysregulation	Peroxisome proliferation (PP): - 37, 50, 54	No, No, No	A fourth study, also finding PP, is summarized; but it is dismissed without explanation, presumably as it occurred at a dose higher than the overall chronic LOAEL.		Three laboratories agree 2,4-D is a weak PP in liver, but Ozaki et al. '01 found more potent PP in rat *kidneys* (2,4-D being *water excretable*). By US-NTP, they used positive control; 3 rodent species);
	53: Decreased metabolism	No			53: Rat, at unknown but "potent" dose.
	5, 6: Oxidative metabolic disorder; reversed by olive oil.	No, No			Supports above PP effect, reversing the effect proves 2,4-D causes it.
	7: Liver tox. at low dose.	No			
Aquatic EcoToxicity	Low dose aquatic ecotox (5, 14, 31, 36, 44).	No, No, No, No, No	"A chronic study on *Pimephales promelas* revealed a NOEC [no effect concentration] of 63.4 mg a.s./L. Two newer chronic studies which showed lower NOEC have been evaluated but they are characterized by limitations."		Studies are not cited, nor are the limitations described. PAN-E's studies show aquatic toxicity at low mg to low u μg per L levels.

Access above-numbered studies at:
www.ncbi.nlm.nih.gov/sites/myncbi/collections/public/14OrOxXWCixKnph-cUjuVPDQ5/

The other 2,4-D studies:

A: Mazhar FM, Moawad KM, El-Dakdoky MH, Amer AS 2012. Fetotoxicity of 2,4-dichlorophenoxyacetic acid in rats and the protective role of vitamin E. Toxicol Ind Health 30: 480-88.

E: Bharadwaj L et al. 2005. Altered gene expression in human hepatoma HepG2 cells exposed to low-level 2,4-dichlorophenoxyacetic acid and potassium nitrate. Toxicol in Vitro 19:603–19.

G: Cenkci S et al. 2010. Evaluation of 2,4-D and Dicamba genotoxicity in bean seedlings using comet and RAPD assays. Ecotoxicol & Environ Saf 73:1558–64.

I: Gonzalez M, Soloneski S, Reigosa SA, Larramendy L 2005. Genotoxicity of the herbicide 2,4-dichlorophenoxyacetic and a commercial formulation, 2,4-dichlorophenoxyacetic acid dimethylamine salt. I. Evaluation of DNA damage and cytogenetic endpoints in Chinese Hamster ovary (CHO) cells. Toxicol in Vitro 19:289–97.

M: Martınez-Tabche L, Madrigal-Bujaidar E, Negrete T. Bull. Environ 2004. Genotoxicity and Lipoperoxidation Produced by Paraquat and 2,4-Dichlorophenoxyacetic Acid in the Gills of Rainbow Trout (Oncorhynchus mikiss). Contam. Toxicol 73:146–52.

O: Rosso SB, Di Paolo OA, de Duffard AM, Duffard R. 1997. Effects of 2,4-dichlorophenoxyacetic acid on central nervous system of developmental rats. Associated changes in ganglioside pattern. Brain Research 769:163–67.

Q: Soloneski S, Gonzalez NV, Reigosa MA, Larramendy ML 2007. Herbicide 2,4-dichlorophenoxyacetic acid (2,4-D)-induced cytogenetic damage in human lymphocytes in vitro in presence of erythrocytes. Cell Biology Internat 31:1316e1322.

de la Rosa P, Barnett JB, Schafer R. 2005. Characterization of thymic atrophy and the mechanism of thymocyte depletion after in vivo exposure to a mixture of herbicides. J Toxicol Environ Health A:68:81-98.

Rosso SB, Cáceres AO, de Duffard AM, Duffard RO, Quiroga S. 2000. 2,4-Dichlorophenoxyacetic acid disrupts the cytoskeleton and disorganizes the Golgi apparatus of cultured neurons. Toxicol Sci:56:133-40.

Amitrole (Aminotriazole, a triazine herbicide)

Assessed by RMS France and Hungary; the acceptable daily intake (ADI) dose for chronic exposures is 0.001 mg/kg a day, unchanged from its original authorization.

	Peer-reviewed toxicity studies found by PAN EU	Found by APPL?	If 'Yes, How Was It Considered? What else was Considered?	RMS Comment & action taken on literature review	PAN Europe comments
Summary	27 studies with important adverse effects were found.	Found: 0% (0/27).	APPL mentioned no open toxicity literature. Below are summary RAR statements justifying their own studies.	"…The applicant provided a literature review report on the toxicology of amitrole. From the registration of amitrole, *none of the published papers gives new/unknown information* compared to data provided in the first monograph."	Yet our search of the same literature ("published studies") for 'amitrole toxicity' in PubMed currently returns almost a hundred published original findings. Given their here-summarized significance, this is an outrage.
Target Organ or System					
Reproductive Toxicity	Lindauer '71	No		Teratogen LOAEL: 40 mg/kg d-	Amitrole is also teratogenic to chickens.
	Gaines et al. '73; Hapke '67 Tjälke '73	No, No, No		Reprotox 7 mg/kg d- but with maternal toxicity—so this industry study not used for the safe daily dose.	These 3 labs all confirm it causes underdeveloped fetuses, (below high dose maternal toxicity).
Endocrine Disruption	Suni et al. '85	No	NOAEL 0.1 mg/kg d- (90 d oral) Hyperthyroid effects in both rat sexes.	Accepted for amitrole's overall chronic ADI of 0.001 mg/kg d-.	LOAEL ~250 mg/kg d-
	USEPA 1995.	No			LOAEL 0.35 mg/kg d- (also thyroid hyperplasia), but no NOAEL established, so likely it is lower than RAR's.
	Fregley '68: decrease in iodide at 0.5 mg/kg d- (NOAEL: 0.1 mg/kg d-).	No	also: 10 ng/L (a very low dose) caused permanent changes in minnow *thyroid* & its gene expression (Li et al. '09, found by appl. & not us). EFSA in previous DAR dismisses it for not being TG-GLP.		Li et al. '09's low dose not discussed. By inhaling, it would be ~ 1 μ g/kg d- TG-GLP were not in existence then—no reason to dismiss. It supports consistent lo dose thyroid risks.
Genotoxicity	11, Chao & Yang '01	No, No			Amitrole synergizes genotoxicity of Cd & Cr6+, by inhibiting protective catalase.
	14	No	"…by weight of the evidence, not [genotoxic]."	"…by weight of the evidence, not [genotoxic]."	- *in vivo* at 200 mg/kg d-
	23, 25	No, No			High--g/L--doses are mutagenic.
	Furukawa at al. '10	No			*In vitro* genotox 20 μM in presence of Cu2+
	Patty's Toxicol. 4:1162	No			Mutagenic in three in vitro tests, one in D/R manner.
Cancer	USNTP Amitrole report.	No		"It was concluded that the thyroid tumours observed in rats were not considered relevant for human *[rats have little T-hormone serum binding protein, leads to high TSH, tissue growth, and hyperplasia and cancer]* and that amitrole does not need to be classified for carcinogenicity."	Ignored much *in vivo* liver cancer evidence. The world's other main carcinogen determiner, US NTP, confirmed (2011) amitrole as a probable human carcinogen; USEPA too. In the mouse--*without the known sensitivity of the rat to thyroid tumors*--both sexes develop thyroid tumors after injection or oral exposure. Amitrole is genotoxic, (also denied by RAR), a cancer factor.
	Innes et al.'69, Napalakov '62	No, No			
	McGregor et al. '94	No			Liver cancers, again oral exposure (potent enough to overcome the known insensitivity of the Innes et al. '69 test).
	10	No			Amitrole also increases the important cancer-promoting protein j38 initiated by the known human carcinogen, Cr6+.
	Johnson '81 (2 yr. rat oral: follicular thyroid cancer at 0.25 mg/kg d- (no NOAEL found).	No	EFSA in previous DAR dismissed study for unspecified "changes in dosing regime".		Precancerous at all doses, cancers also increased. Consistent with other thyroid cancer indications.
Synergistic Toxicity	(11), (Chao & Yang '01)	(No), (No)			Amitrole synergizes both cadmium and chromium genotoxicity.
	(Suni et al. '85)	(No)			Thyroid hyperplasia when synergized with N-nitrosobutylurea
Neurologic Toxicity	-		-	-	NO DATA found by anyone.
Immune Toxicity	-		-	-	NO DATA found by anyone.
Oxidative Damage It reliably, potently inhibits catalase; = less hydrogen perox, & oxid damage.	15 16 17 12 18 20	No No No No No No			Oxidative damage *in vitro* heart cells. A man's inhalation: similar lung damage. …this mechanism confirmed in both heart & lung cells, *in vitro and in vivo.* Similarly, eye cataracts by amitrole … … confirmed via enzyme mechanism.
Aquatic Toxicity (most ecotox studies are on aquatic organisms)	5, 7	No, No	Fish LC50: 100 mg/L; also: 10 ng/L caused permanent changes in minnow *thyroid* (Li et al.'09, noted by appl.); Invertebrate LOEC: 560 ug/L	Data gaps claimed despite RAR reporting these findings.	Frogs delays devpmnt, teratogn 10 μg/L Mammalian tox. studies have data gaps noted by RAR yet an ADI proceeds; but this false claim of data gap (a v. low dose study was ignored!) means no fish RA (only a use limit, assumed stringent).

Access above-numbered studies at: www.ncbi.nlm.nih.gov/sites/myncbi/collections/public/14ypne_wwo8fq-XWFnLXO9R5a/

The other Amitrole studies:

Chao JI, Yang JL 2001. Alteration of cadmium-induced mutational spectrum by catalase depletion in Chinese hamster ovary-K1 cells. Mutat Res 15 498:7-18.

Fregly MJ 1968. Effect of aminotriazole on thyroid function in the rat. Toxicol Appl Pharmacol:13:271-86.

Furukawa A et al. 2010. Oxidatively generated DNA damage induced by 3-amino-5-mercapto-1,2, 4-triazole, a metabolite of carcinogenic amitrole. Mutation Res:694:7–12.

Gaines TB, Kimbrough RD, Linder RE 1973. The toxicity of amitrole in the rat. Toxicology and Applied Pharmacology:26:118–29.

Hapke HJ. Toxicity of aminotriazol for domestic animals [from German]. Zentralbl Veterinarmed A:469-86 1967.

Innes JRM et al. 1969. Bioassay of Pesticides and Industrial Chemicals for Tumorigenicity in Mice: A Preliminary Note. J Natl Cancer Inst:42:1101-14.

Johnson WD, Becci PJ, & Parent RA (1981) Lifetime feeding study of amitrole in Fischer 344 rats. Waverly, New York, Food and Drug Research Laboratories, Inc. (Unpublished report No. 5651).

Lindauer, W., Salam, N., Sopher, D. The herbicide 3-amino-1,2,4-triazole (amitrol) as teratogen. Environ. Res. **4**, 539 (1971).

McGregor DB, Pangrekar J, Rosenkranz HS, Klopman G 1994. A reexamination of the low prevalence of carcinogens in an early carcinogen screen. Regul Toxicol Pharmacol:19:97-105.

Napalkov NP 1962.. Blastomogenic action of 3-amino-1,2,4-triazole [Article in Russian] Gig Tr Prof Zabol:6:48-51.

Patty's Toxicol 5th Ed. 2000. Edited by: Bingham E et al.

Sumi C, Yokoro K, Matsushima R 1985. Inhibition by 3-amino-1H-1,2,4-triazole of hepatic tumorigenesis induced by diethylstilbestrol alone or combined with N-nitrosobutylurea in WF rats. J Natl Cancer Inst:74:1329-34.

Tjälve H 1974. Fetal uptake and embryogenetic effects of aminotriazole in mice. Arch Toxicol 25. XI 33:41-8.

USEPA 1995 Amitole Reregistration Eligibility Document, p.12: http://epa.gov/oppsrrd1/REDs/0095red.pdf

USNTP (National Toxicology Program). Amitrole: http://ntp.niehs.nih.gov/ntp/roc/twelfth/profiles/Amitrole.pdf

Fenhexamid (a hydroxy-aniline fungicide)

RMS UK's proposed chronic ADI is 0.2 mg/kg a day, based on a NOAEL of 19 mg/kg d- (LOAEL was 137 mg/kg d-); all unchanged from previous 2001 authorization.

	Peer-reviewed toxicity studies found by PAN EU	Found by APPL?	If 'Yes, How Was It Considered? What else was Considered?	RMS Comment & action taken on literature review	PAN Europe comments
Summary	Only 13 toxicity studies were found by PAN-E; none on chronic mammalian exposure (despite availability to academia for some years).	11 % (1/9)	APPL dismissed the one study of ours that they found. "A search of scientific peer-reviewed open literature on fenhexamid & its metabolites within last 10 years revealed 616 references"	"It was concluded from the abstracts of the [open literature] papers that *they contained no information which would have an impact on an endpoint* or would result in the need to adapt a risk assessment..."	Surprisingly (long-time market) almost no toxicity investigation by academia. The Endocrine Exch. (TEDx) searched other databases, also found none. But APPL failed to find what does exist.
Organ/System					
Reproductive Toxicity				[The reproductive/teratology studies considered showed no effects below 1000 ppm in feed]	Academia data gap.
Endocrine Disruption	2, 4, 6 3	No, No, Yes No		"…submission fenhexamid has been tested in vivo up to very high doses in comprehensive toxicological studies. Without any indication for endocrine mediated effects in these *in vivo* studies the positive result reported…is considered to have no relevance.."	- *In vitro* ED (these three at high doses) - *In vitro* ED at both 10-100 nM (3-30 ug) and at 10 μM (3 mg); open literature. No in vivo: an academia data gap. This is incorrect—the low doses at which ED occurs were not tested;
Genotoxicity				[All *in vitro & in vivo* results negative.]	Academia Data Gap.
Cancer				In combined chronic tests there were no cancer effects, other effects only at high doses. For the ADI the overall LOAEL was 137 mg/kg d- and NOAEL was 20 mg/kg d-.	Academia Data Gap.
Synergistic Toxicity	(4)	(No)			Response increased when combined with other pure anti-androgens.
Neurotoxicity				no acute tox (no data for develop'l tox)	Academia Data Gap.
Immune Toxicity					Academia Data Gap.
Ecologic Toxicity	1 7 8 10 11 13	- No No No No No	Too new for APPL to have found.		LOAEL 20 μg/L - LOAEL 20 μg/L (oxidative damage) LOAEL 20 μg/L (damaged symbiosis) - LOAEC 10 mg/L

Access above-numbered studies at:
www.ncbi.nlm.nih.gov/sites/myncbi/collections/public/1j70ZYvObsw8keakGl2fNJuAT/

Lambda (λ)-Cyhalothrin (pyrethroid insecticide)

Sweden and Spain RMS proposed chronic exposure ADI is 0.0017 mg/kg a day, based on the same toxicity study from its original authorization on 1-1-2002; but with a new 3-fold safety factor added for developmental neurotoxicity, as the RMS believe is unknown. Cyhalothrin is a mix of four isomers. Gamma (y)-cyhalothrin--also a pesticide--is the most insecticidal (potent) isomer. This RAR, for lambda (λ, or l)-cyhalothrin, has equal parts of two isomers, including the potent y-cyhalothrin.

	Peer-reviewed toxicity studies found by PAN EU	Found by APPL?	If 'Yes, How Was It Considered? What else was Considered?	RMS Comment & action taken on literature review	PAN Europe comments
Summary	33 l-cyhalothrin studies with important adverse effects, most at low dose, were found by PAN-Europe.	36% (12/33) Found *(not counting new one. Multiple effect ones not double counted noted by (brackets).*	100% of these were dismissed by APPL APPL for most endpoints says, e.g.: "*…a literature search & evaluation of open sources was conducted by applicant TFL describing influence of l-cyhalothrin on reproductive parameters. Few publications were found during literature search, all…were considered inadequate.".*		Unlike other APPL, some effort to discover/discuss academic findings… but APPL missed two thirds of the 32 we deemed especially relevant (selected from about 100 published toxicity studies).
Organ/System					
Reproductive Toxicity	3	No	"Based on available relevant data..[is] not.. repro[tox,] ..not embryotox. or teratogenic."	RMS had no judgements to make as all studies mentioned had higher NOAELs than the ADI one.	These hypothetical reasons for excluding academic studies-are never tested.
	26	Yes	"...[that] effects [were] caused by co-formulants cannot be excluded."		Seminal vessel & sperm toxicity at 0.2 mg/kg d- rats, oral (and no NOAEL found). Even lower than RAR's ADI NOAEL Supports above tox, LOAEL 15.383 mg/kg d- (no NOAEL found)
	56 developmental	No			
	60 developmental	Yes			
	65, 66 developmental	Yes, Yes	"..effects [may be] caused by co-formulants" "..effects [could be] due to the vehicle"		N/LOAEL(unclear which) 6.3 mg/kg d- LOAEL 63 mg/kg d- (no NOAEL) LOAEL ~5 mg/kg d-
Endocrine Disruption	21, 24, 33, Akhtar et al. '96 (all *in vitro).*	Yes, Yes, Yes, No	"…[*in vitro* and] not performed according to current validated methods and guidelines for testing of chemicals and thus less reliable. The re-evaluation of existing toxicology studies (submitted) did not show indications of an endocrine effect *in vivo.* The…reproductive…and thyroid [alterations]...ends up in an inconsistent overall picture and not a pattern suggesting endocrine disruption. Any findings for endocrine activity should have been visible in a clear, consistent and to some extend dose dependent manner, which was clearly not found. The differences between the findings of i*n vivo and in vitro* studies can be explained by differences in metabolism and pharmacokinetics between the both types of studies. In conclusion, the findings of the in vitro studies do not qualify lambda-Cyhalothrin for an endocrine disrupter "..concentrations …were about two orders of magnitude higher than positive control … estradiol. ...at th[at, low] concentration,...was about 3 x less effective than estradiol."	"RMS recommends that the potential … is reconsidered when EU harmonised guidance is established based on the work & final conclusions of the EC work on defining criteria to identify endocrine disrupting substances."	For irrefutable biologic reasons, endocrine disruption does not occur in a linear dose/response (e.g. Laura Vandenberg et al.'s 2013 review). Risk assessors continue to deny the realities of biology. So this APPL's claim of no EDC-ness cannot stand because they disavow the results of low dose tests. Yet the in vitro findings (most found by this Notifier) proves that in vivo low-dose tests (by academia) are needed.
Genotoxicity	Fahmy & Abdullah 2001	No	"All studies were performed in accordance with ICI policy for Good Laboratory Practice[]. …reinforced with an UDS test and an in vivo mouse micronucleus test. The results were consistently negative and based on these data, it was concluded that lambda-cyhalothrin does not possess any mutagenic or clastogenic properties." (too new)	"…results were consistently negative.... concluded that lambda-cyhalothrin does not posess any mutagenic or clastogenic properties." "No genotoxic effects… observed in the standard in vitro test package …reinforced with additional UDS test and in vivo mouse micronucleus test."	Why does RMS bother re-arranging the Notifier's phrases? Their conclusion is directly contradicted by published studies which the Notifier failed to find:
	1	-			Cytogenic effects in mice, dose unknown
	2	No			-
	48	No			Vivo LOAEL 6.23 mg/kg d- no NOAEL
	59	No			Vivo NOAEL 0.8 mg, LOAEL 3.06 mg/kg d-
	62	No			Vitro LOEC 5 ng/L Vitro LOEC 1 ng/L; no NOAEL found.
Cancer	None		"The increased…mammary adenocarcinoma [at 10.58 & 50.7 mg/kg d-] developed late in the study with no pre-neoplastic changes (only lesions) and the effects were similar to both the experimental and historical controls." "At ECCO meeting 7 (1997) it was concluded that the weight of evidence (including the negative genotoxicity data) was that there was no carcinogenic potential for lambda-cyhalothrin. There are no new data…"	"No carcinogenic potential."	A few other pyrethroids are animal carcinogens in open literature studies, so a similar study (including dermal exposure) is needed for cyhalothrin). …Yet notifier also says these tumor rates were above those in historic controls. Contrary to both Notifier and RMS, it is genotoxic, including to blood cells.
Synergistic Toxicity	16 in vitro	No			Liver damage in a mix w/ organo-phosphates; doses unknown.

	Peer-reviewed toxicity studies found by PAN EU	Found by APPL?	If 'Yes, How Was It Considered? What else was Considered?	RMS Comment & action taken on literature review	PAN Europe comments
Developmental Neurotoxicity (DNT)	8, 9	No, No	"...unknown vehicle…effects observed [could be] due to the vehicle."	RMS reviews DNT studies and find there are no relevant ones, so an extra 3-fold safe factor is added to the ADI; presumab;y instead of ordering a study.	RMS, APPL missed important DNT findings. Both LOAELs 1 mg/kg d-; no NOAEL.
	13	No			NOAEL 0.1 mg/kg d-
	67	Yes			LOAEL~40 mg/kg (200 ppm in water); no NOAEL found
Other Neurotoxcity	35	No			10 mg/kg d- LOAEL; no NOAEL
	43	No			NOAEL 0.52mg/kg d-; LOAEL 1.32mg/kg d-
	52	No			LOAEL 8 mg/kg d- (no NOAEL found)
	58	No			NOAEL 1 mg/kg d-(LOAEL 3mg/kg d)
Immune Toxicity	20	No		"…some *in vitro* studies from the open literature that [it]…may affect the immune system…However, in the available standard toxicity studies there was no indication of immunotoxicity."	In fact, they are *in vivo*!: 4 mg/kg d-LOAEL; NOAEL1 mg/kg d-
	29	No			1 mg/kg d- LOAEL (no NOAEL);
	47	No			1 mg/kg LOAEL, 0.6 mg/kg d-NOAEL
	50	No			LOAEL 0.8 mg/kg, no NOAEL found
	57	No			LOAEL 0.8 mg/kg, no NOAEL found
Oxid. Damage	16	No			*In vitro.*
Aquatic Toxicity (the majority of ecotox studies.)	17	No			LOEC 0.1 ng/l (and no NOEC found)
	22	Yes	"…nitrite can have toxic effects on aquatic invertebrates as well, even at lower concentrations. However ...indicate that most of this nitrogen should belong to the nitrate fraction, thus leaving nitrite at merely nontoxic concentrations, having no negative influence on the animals used for the tests."	"No guideline was followed since the test is a non-standard toxicity test. Although the RMS does not consider the effects investigated to be relevant for an endpoint derivation ...the study will be considered as supportive information."	LOEC 1 ng/l (and no NOEC found). The methods for aquatic invertebrate tests are variable enough that 'lack of standardization' is a not a valid criticism. In fact, as Notifier summarized, these were through, precise and transparent experiments, making it a relevant LOEC; supported by similar independently derived NOECs below.
	28	Yes	[but not mentioned.]	[not discussed.]	LOEC 1.05 ng/l (and no NOEC found)
	36	Yes	[No APPL mention found, but we will assume that the RMS discussion resulted from APPL's noticing it].	"No guideline was reported to be followed but the study generally seems to follow OECD 235, Chironomus sp., Acute Immobilisation Test and EPA OPPTS 850.1735 …The test dilution water was taken from different ponds and it is unclear if these were tested for chemical residues prior to test. In addition, the study was conducted on a formulation not representative of the formulations subject for renewal. Due to uncertainties in test conditions, the RMS does not think an endpoint useful for risk assessment can be derived from the study. …This study will be considered as supportive information."	LOEC acute 1.4 ng/l (no NOEC found)
	44	Yes	"The animals…were collected in the wild. …it is known that parasitism can significantly alter their drift behaviour (especially of Gammarus). …It is not stated … whether the test animals have been investigated for parasitation nor have the inherent drift rates been stated. …potential parasitation might account for at least a part of the increase of drift behaviour when it comes to quantification."	"…The study is not considered valid for the derivation of an endpoint useful for risk assessment due to potential parasitation and study being conducted on a non-representative formulation."	LOEC 1 ng/l (no NOEC found)
	Parthasarathy & Joseph 2011	No			0.3 μg/L & 1.1 μg/L

Access below-numbered studies at: **www.ncbi.nlm.nih.gov/sites/myncbi/collections/public/1hUpepCT87doXLcNQG-dvAjA3/**

The other l-Cyhalothrin studies:

Akhtar N, Kayani SA, Ahmad MM, Shahab M. Insecticide-induced changes in secretory activity of the thyroid gland in rats. J. Appl. Toxicol. 1996;16:397-400.

Fahmy MA and Abdulla EF 2001. Cytogenetic effects induced by the natural pyrethrins and the synthetic lambda cyhalothrin in mice in vivo. Cytologia 66:139–49.

Parthasarathy R, Joseph J 2011. Study on the changes in the levels of membrane-bound ATPases activity and some mineral status in cyhalothrin-induced hepatotoxicity in fresh water tilapia (Oreochromis Mossambicus). African J Environ Sci & Technol. 5:98-103.

Thiabendazole (a post-harvest fungicide)

Also long-approved for veterinary anti-helminthic (worms). RMS Spain and Netherlands say: "For the previous submission the ADI value was established based on the two year rat study with the safety factor of 100. This data was supported by human data and would result in the same ADI when using a NOAEL of 3mg/kg/day and a safety factor of 25 (because only males investigated). Thus, ADI established was 0.1 mg/kg b.w/day."

	Peer-reviewed toxicity studies found by PAN EU	Found by APPL?	If 'Yes, How Considered? What else was Considered?	RMS Comment & action taken on literature review	PAN Europe comments
Summary	65 studies by academia with important adverse effects (some at low dose)	APPL found: 2% (1/65) Multiple effect studies not double counted).	100% (one found in list of studies but not discussed).	Co-RMS Spain and by implication the APPL, make the novel claim that the new mandate to perform a literature search is voluntary, relying on EFSA Guidance. No other RAR we have seen so argues.	With over a hundred published original toxicity findings (from which we selected 65 as especially relevant), APPL had a treasure of data to find.
Organ/System					
Reproductive Toxicity	In vitro: 41. In vivo: 37, 49. Three neg. findings: 17, 38, 43 (two by Merck company).	N N, N N, N, N		"…In rabbits a very slight increase in the incidence of hydrocephaly was found at toxic doses... However, this finding could not be reproduced in a subsequent study in this species. Therefore, thiabendazole is not considered to adversely affect development at non-materno-toxic dose levels in rabbits. In…the mouse, thiabendazole showed maternal toxicity, not teratogenicity effects, at 100 and 200 mg/kg/d (decreased bodyweight gain and food consumption). NOEL for maternal and developmental toxicity of thiabendazole in the mouse was 25 mg/kg/d. ...no developmental anomalies associated with oral [anti-helmithic] treatment."	This typical result of regulatory developmental toxicity is at odds with the extensive findings by financially disinterested academics, incl. 61 which falsifies the claimed LOAEL with a 26,4 mg/kg d- LOAEL (no NOAEL found) #46: teratogen at a *single* 1 g/kg dose.
	Teratogenicity: 64, 60, 61, 56, 53, 54, 58, 59, 55. And 47, the only neg. one, is just in vitro.	N, N, N, N, N, N, N, N, N N			
Endocrine Disruption	In vitro: 7, 11	N, N		Co-RMS cite #36 in calling for Notifier to perform more study of possible endocrine toxicity.	
Genotoxicity	9, 36, 13, 33, 10, 12, 2 (one neg. finding: 6).	N,N,N,N,N,N,N N		"All of these new [RAR]…studies were negative and add to the weight of evidence that thiabendazole is not genotoxic."	Many academic labs falsified RAR's claim; APPL never found one!
	In vivo: 21	N			LOAEL 200 mg/kg d- "in var. organs"
	Aneuploidy (extra chromosones) 3, 26, 40, 30, 29, 23, 28, 8, (41), 19, 39	N,N,N,N,N,N,N,N,(N),N, Yes.	65: Listed, no discussion.	"…shown to produce non- disjunction and aneuploidy in fungi and mammalian cells. These …are about an order of magnitude above …toxic dose levels in vivo and so are not achievable in the whole animal. ...supported by ...uniformly negative …in vivo in rats and mice at toxic dose levels."	Contradicting this assessment are two in vivo (# 27 & 39). #65 is listed, not discussed. #3: 0.5 μg/ml.
Cancer	57, (49). Also, hyperplasia (preceeds cancer) at high dose: 14, 16, 20, 25, 32, 44.	N, (N) N,N,N,N,N,N		"…transient decrease in thyroid hormone results in increases in thyroid stimulating hormone (TSH) ..a growth promoting effect on the thyroid and increase the incidence of benign thyroid tumors in this species. This mechanism does not occur in humans and, therefore, these findings are not considered relevant. …In other completed study in rat doses > 90 mg/kg/day resulted in significant decreases in thyroid hormone and increases in TSH and thyroid follicular cell hyperplasia which were completely reversible following cessation of treatment. These findings indicate that thiabendazole affects the thyroid in rats indirectly by increasing the clearance of thyroid hormone. NOEL for effects on thyroid hormone and thyroid tumour incidence of 10 mg/kg/day has been clearly established."	This assessment is somewhat contradicted by the missed results from academia.
Kidney, Bladder & Liver Toxicity	1, 48, 45, 24, 45, 52, (14, 16, 20, 25, 32, 44).	N,N,N,N,N,N, (N,N,N,N,N,N).			Clearly (!) it causes reactive damage when metabolized & cleared from liver, kidneys and bladder – all missed!
Neurotoxcity				"…The [semi-chronic neurotoxicity] NOAEL was established in 750 ppm (95 mg/kg/day, (m) and 108 mg/kg/day (f)) based on decreases in absolute and relative bodyweights and decreases in food consumption seen at 1500 ppm."	No chronic neurotoxicity (including developmental) study done by APPL (in addition to a data gap by academia).
Immune Toxicity	4, 62.	N, N		"…A 28-Day Dietary Immuno-toxicity Study in Mice was carried out … to support the review…in the US. …data indicates a depression of antibody response in mice administered …1027.0 mg/kg bw/day with a NOEL for the ...humoral immune response…of 205.6 mg/kg bw/day."	#62: a human case study. #4: In frogs (vertebrates), it induced strong immune reactions for all exposure periods at low concentration of 0.7 μg/L.
Aquatic/Ecologic Toxicity	(4, 16). 63	N		"...note[] that Thiabendazole has been used safely in breeding sheep, horses, goats, and cattle as an anthelmintic for many years with no developmental anomalies associated with oral treatment."	#40: Dead cattle after high dose anthelmintic (i.e. worm cure) contradict that.

Access above-numbered studies at:
www.ncbi.nlm.nih.gov/sites/myncbi/collections/public/1LS4P8zcoflUhVm_R--JAB2QE/

Esfenvarelate (Pyrethroid insecticide)

RMS UK and Portugal say Esfenvarelate's proposed ADI of 0.0175 mg/kg d-, based on an *acute* neurotoxicity rat study (unusual in regulatory toxicology based on dose-ranging, none of the sub-chronic or chronic exposure doses showed toxicity at lower does); this is slightly lower than the previous DAR.

Note: This RAR makes irregular and unclear use of read-across to studies on its parent molecule, fenvarelate ('fen', a long-time authorized pyrethroid insecticide—over time its most potent isomer, esfenvarelate ('esfen'), was able to be purified and then authorized for use). *Academia's published literature on fen is large and shows great potency*, and it can be assumed (though uncertain) that some fen toxicity findings are due to its ~20% esfen content. There is a decent academic toxicity literature on esfen, so the uncertainty of read-across is unneeded, but as they overlap so much; we as the APPL did, read-across to fen (though only for the most potent findings).

	Peer-reviewed toxicity studies found by PAN EU	Found by APPL?	If 'Yes, How Considered? What else was Considered?	RMS Comment & action taken on literature review	PAN Europe comments
Summary	We saw 153 published studies of some interest (including many on fen toxicity); ~50 show notable lo-dose toxicity of esfen.	1 of c. 130 found…	…and it was not discussed.	RMS seems to have made no change to APPL Sumitomo Europe's conclusions!	Once again an APPL fails to meet the mandate; this time ignoring a huge trove of toxicity findings.
ORGAN/ SYSTEM:					
Reproductive Toxicity	13: read-across: Just 0.1875 mg/kg d- fen. 48 " - ": 64 umol/L fen M reprotox rat. 84, 8: Reprotox to fish: Fen lo-dose vivo, vitro	No No No, No		"No reproductive/developmental toxicity observed".	One esfen & two read-across from fen (supported by 2 unlisted occupational fen expos sperm damage) indicate RAR wrong.
Endocrine Disruption	Pine et al. '08: Suppresses diurnal LH rise, Delays puberty in F rats.	No		Not tested.	Several fen read-across ones not found; the one esfen EDC finding ignored.
Synergistic Toxicity	10, 21, 38, 52, 57, 74.	None		Not tested.	Surprising number of synergistic toxicity findings—most ecotox.
Genotoxicity	Read-across fen: 16, 86, 126, 127, 130, 131.	None		"No genotoxicity elicited".	Again, read-across hints that RAR is wrong.
Cancer	Promotion of cancer in vitro: 111 Read-across to fen: 150.	Yes No	Not discussed.	"No indications of cancer": "Elevation of cancers by Fen (read-across) were not caused by the exposure, according to independent experts".	The single study of ours APPL found (not discussed) is (unusually) by APPL=so their claim of no carcinogenicity is contradicted by their own work!! (albeit *in vitro*). This may be the un-named read-across study where the RAR says fen was not the cause.
Kidney, Bladder & Liver Toxicity	Read-across to fen: liver tox at 0.75 – 3.0 mmol/kg d-: 95.			No chronic toxicity to any organs in any of the tests—only high-dose decrease body wt.	
Neurotoxcity	~~31: 0.0625 ug/L; neurotox fish.~~ ~~42: 0.01 ug/L immune, neurotox in fish~~ ~~43: Mech. of developmental neurotox.~~ 54: Rat neurtox.	~~No~~ ~~No~~ ~~No~~ No		RMS concur with APPL that acute neurotox at 1.75 mg/kg d- is most reliable NOAEL for ADI.	Fish only vertebrates, but very low dose neurotox (& other) confirms: NOAEL 0.45 mg/kg d-, falsifying LOAEL.
Immune Toxicity	(42)			Not tested.	
Aquatic/Ecologic Toxicity	1, 5, 7, 9, (10), 14, (21), 23 (Fen but lo-dose), (31), 36, (38), 39, 40, 41, (42), 44, 47, (52), 56, (57), 75, (78), 83, (84), 91, 92 (ind study!), 102, 104, 126. Persists in environment: 71.	\ None / No			As for all pyrethroids, a large number of aquatic toxicity studies all showing very low dose (most at ng/L) toxicities -- all ignored!

Access above-numbered studies at **www.ncbi.nlm.nih.gov/sites/myncbi/collections/public/14ypne_wwo8eq-X4ElIlJeykh/**

The other Esvenvarelate study:

Pine MD, Hiney JK, Dearth RK, Bratton GR, Dees WL 2006. IGF-1 administration to prepubertal female rats can overcome delayed puberty caused by maternal Pb exposure. Reprod Toxicol:21:104-9.

Glyphosate (Herbicide)

Rapporteur Member States (RMS) DE and SK have released a draft revised assessment report (RAR) for Glyphosate re-authorization in the EU. The open peer-reviewed literature must now be taken into account (EU Regulation 1007/2009). Glyphosate's alleged draft safe chronic exposure dose, the ADI, remains at 0.5 mg/kg d-.

Note on formulations: Glyphosate is the world's most utilized herbicide (including demand spurred by glyphosate-tolerant crops), and as the surfactants in its various formulations (including "RoundUp") are quite toxic--academics have *~four times as many toxicity findings of the applied product as they have for active substance glyphosate*. Nevertheless we identified many published findings of glyphosate toxicity to check if APPL had found these, as required. Frequently APPLs imply that toxicities found for formulations are due to the surfactant…but ***never determine if that is so***! We do include a small handful of interesting (low dose or cancer) *formulation* findings, as these should prompt further studies to see if glyphosate is responsible for such serious effects–note that the RAR also discusses studies that tested only formulation.

	Peer-reviewed toxicity studies found by PAN EU	Found by APPL?	If 'Yes, how considered? What else Considered?	RMS Comment & action taken on literature review	PAN Europe comments
Summary	Of 243 toxicity studies we initially flagged, we found 146 glyphosate toxicity findings (~10 of our 146 studies are not listed in RAR's tables of published literature, but are discussed).	52% Found* (76 of 146); but just 31% discussed. Some 'N' may have been ID'd by APPL in prev. RARs.			Just 31% (31 of 76) of our studies that were found were discussed, less than for other RARs.
ORGAN/ SYSTEM:					
Teratogenicity	93, (169).	Y	"…highly artific[i]al routes of exposure [&] excessive doses. Craniofacial malformations weren't noted in [APPL's] developmental studies in rats or rabbits"		93: teratogen to chicken & frog embryos @ 1/5000 dilution. Their publ. response to such criticsm is not in RAR. In brief, they and a review (Antoniou et al 2012) explain the dose route & level relevance. That it is teratog. In 3 species in two academic studies using sensitive methods; plus very similar results using RUp, argue it is APPL's insensitive tests that give false negative results.
Reproductive/ devlopmental Toxicity	25 rat, 76 rat, (93),106, 151, 219 Vitro: 74, 213	N, Y, Y, Y, Y, N, Y	Never discussed: 151. Heavy criticism: 76 & 106(RUp):.	76, 106: dosing shorter & began after OECD TG calls for. Poor reporting, incl. for litter eff. which can confound ED. Several different results of the two studies "of particular concern".	25: 1/250th LD50. 106: lo dose 5 mg/kg d- but RUp. 76 & 106: authors dose timing designed to catch hypothalamic sex differentiation, whereas 106 used USEPA protocols for ED tests and tested RUp; accounting for the inconsistencies between the two. Both conformed w/ Endoc. Soc recommendations; unlike OECD TG, which may account for the M reprotox ED tox found @ the RAR's alleged NOAEL of 50 mg/kg d-.
Endocrine Disruption	In Vivo: 4, 6, (25), 35, (76), (106), 127, 129, Wikvall '01.	N, N, N, Y, Y, N	Never discussed: 127, 129.		6: in snail. 106: 5 mg/kg d- of RUp, M reprotox, ED.
	In Vitro: 16, (74); 112, 137, 144, 163,	N, Y, Y, Y, Y	137: dismissed as "homeostasis".		16: 0.036 g/L. 74: 1 ppm 137: No, ED gene expression is a flag to investigate. 163: E/T disruption at non-cytotoxic dose. Cell prolif. via ER at 10(-12)M (169 pg), < human body levels
	Schuster "11.	N			
	Thongprakaisang et al. '13,	Y	T' 13: Lo dose not discussed.		
Synergism	(24), (25), (66), (125), (184).				25: reprod, 184: genotox; others: ecotox.
Genotoxicity	In Vivo: 60 lo dose fish, 113 rats, 124,(151), Prasad et al'09	N, Y, Y, Y	Heavy criticism: 24, 122. Never discussed: Prasad et al. '09, 68, 103, 132, 149, 155, 156, 184.		60: at 18 ug/L 113: 1/250th LD50. 122: at 1/10,000 dilution:-gen. population body level.
	In Vitro: 3, 39, 42, 47, 52, 61, 64, 68, 72, 103, 115, 117, 122, 132, 149, 155, 156, 170, 184, 207, 213, 214, 215, 222, 223.	N,N,Y,Y,N,Y,Y,Y,N,Y, Y,Y,Y,Y,Y,Y,N,Y,Y,N, N,Y,N,Y.			

	Peer-reviewed toxicity studies found by PAN EU	Found by APPL?	If 'Yes, how considered? What else Considered?	RMS Comment & action taken on literature review	PAN Europe comments
Cancer	*In Vivo*: 100	Y	RoundUp used. A biochemical response, not toxicity. Results variable so no cancer significance.	RMS and APPLs dismiss many APPL findings of salivary gland toxicity as "adaptive", and highlight inconsistent APPL findings.	100: Abstract indicates glyphosate tested (unusual if scientists would misname it). It says the protein expression measured is very significant to cancer.
	225	Y	Brief discussion but cancer part of the study not mentioned.		225: The US NTP finds salivary gland lesions after hi dose glyphosate just a year after US EPA classes glyphosate as 'probably not carcinogenic'.
	De Roos et al. '05	Y	Industry's published letter is described.		This prospective study's authors' very specific co-published rebuttal is not even mentioned!
	Hardell et al. '02	Y	Association based on small numbers; unquantified exposure, uncontrolled co-variates, and unconfirmed in the multivariate regression.		In fact a meta-study (515 cases, 1141 controls) with validated exposure estimates (always proven to be accurate enough) and some co-variates controlled More important, APPL's own studies found high dose lymphomas at elevated rate if studies using same CD1 mouse strain were summed; supporting the same finding in Swiss albino mice. In support are 3 (here uncited) case-control lymphoma associations (prompting de Roos et al. association with other immune cancer) and Hardell's meta lymphoma finding. In sum, converging lines of evidence coherently if incompletely point to a lymphoma risk.
	In Vitro: (52), (64), 69, (72), 101, (Thongprakaisang '13).	Y, Y	101: not discussed		
Metabolic Toxicity	*In Vivo*: 11, (97), 167, 177, 239, 242, Hietanen et al. '83.	N, Y, Y, N, N, N	167: "..due to dehydration or diet?" 177: ""toxicity due to surfactant"		97: fish liver tox, "envirn'ly realistic dose". 177: 4.87 mg/kg d- but RUp: liver tox. APPL has no proof of claim result is due to surfactant.
	In Vitro: (170), 241,Herrmann&Weaver'99, Zhao et al. '98	N, Y, N			
Neurotoxicity	*In Vivo:* 43, 79, 200.	N, Y, Y	Not discussed: 79, 198. 200: "..due to dehydration or diet?" &: "unrealistic high doses"(!).		200: Developmental neurotox (dose unstated in abstract).
	In Vitro: 28, 198.	N, Y			
Oxidative Damage	41, 81, 212 (bleeding), 235 (dermatologic)	N, Y, N, N	81: not discussed.		41: 0.7 mg/L in water (c. 0.2 mg/kg d-): anti-oxidant enzyme disturbances in rats.
Immune Toxicity	*In Vivo:* (127), (129). *In Vitro*: 29, 37, 190, (207).	Y, Y, Y	29 & 37: heavily criticized. 190: not discussed.		
Aquatic/Ecologic Toxicity	Lo-dose: 18, 24, 36, 38, 56, 87, 97, 125, 131, 143, 150, 176, 196.	N,N,N,N,N, Y,Y,Y, Y,N, Y, Y,Y	Never discussed: 125, 176, 196.		36: 0.05 mg/L aq. ecotox (D. magna) 125: Aq. ecotox at 2 ppb.
	Other: 12, 15, 19 & 44, 30, 31, 32, 54, 57, 65, 66, 75, 84, 89, 90, 91, 95, 98, 99, 105, 109, 111, 114, 116, 118, 119, 120, 121, 130, 134, 138, 139, 140, 146, 154, 161, 165, 169, 171, 180, 191, 203, 205, 206, 210, 216, 217, 227, 229, 230, 234, 236;	N,N,N,N,N,N,N,N,N,N, N,Y,Y,Y,Y,N,N,Y,N,Y,Y,Y, N,Y,Y,N,N,Y,Y,Y,Y,Y,Y,Y, N,Y,Y,Y,N,N,Y,N,N,Y,N, N,N,N,N,N,N,N,N	Never discussed: 75, 91, 109, 111, 114, 118, 121, 130, 134, 165, 169, 191, 206.		
	Vitro: (28), 240.	N			
Exposure, PBPK	166, 110; 221.	N, Y; N			
Unknown Effect	Feinchemie Schwebda, AG	N			In '98 DAR: chronic NOAEL 5 mg/kg d- (LOAEL unknown).

Access above-numbered studies at:
www.ncbi.nlm.nih.gov/sites/myncbi/collections/public/1IoH85XekdSXuno_Xemk0jK5h/

The other Glyphosate studies:

De Roos AJ et al. 2005. Cancer incidence among glyphosate-exposed pesticide applicators in the Agricultural Health Study. Environ Health Perspect:113:49-54.

Hardell L, Eriksson M, Nordstrom M 2002. Exposure to pesticides as risk factor for non-Hodgkin's lymphoma and hairy cell leukemia: pooled analysis of two Swedish case-control studies. Leuk Lymphoma:43:1043-9.

Herrmann KM, Weaver LM 1999. The Shikimate Pathway. Annu Rev Plant Physiol Plant Mol Biol:50:473-503.

Hietanen E, Linnainmaa K, Vainio H 1983. Effects of phenoxyherbicides and glyphosate on the hepatic and intestinal biotransformation activities in the rat. Acta Pharmacol Toxicol (Copenh):53:103-12.

Prasad S, Srivastava S, Singh M, Shukla Y 2009. Clastogenic effects of glyphosate in bone marrow cells of swiss albino mice. J Toxicol:2009:308985.

Schuster, I. Cytochromes P450 are essential players in the vitamin D signaling system. Biochim. Biophys. Acta 2011, 1814, 186–99.

Thongprakaisang S1, Thiantanawat A, Rangkadilok N, Suriyo T, Satayavivad J. 2013. Glyphosate induces human breast cancer cells growth via estrogen receptors. Food Chem Toxicol:59:129-36.

Wikvall, K. Cytochrome P450 enzymes in the bioactivation of vitamin D to its hormonal form (review). Int. J. Mol. Med. 2001, 7, 201–9.

Zhao J, Williams CC, Last RL 1998. Induction of Arabidopsis tryptophan pathway enzymes and camalexin by amino acid starvation, oxidative stress, and an abiotic elicitor. The Plant Cell;10:359-370.

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