Roundup causes non-alcoholic fatty liver disease

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Current Acceptable Daily Intake (ADI) / chronic Reference Dose (cRfD)

- EU/Aus/NZ: 0.3mg/kg/day
- China/Russia: 1mg/kg/day
- USA: 1.75mg/kg/day

➢ Are these exposures really safe?
Our conclusion: ADI should be at least 3-fold lower
Glyphosate: multiple toxic effects

- Antibiotic
- Nutrient metal chelator; e.g. Mn, Mg, Zn, Co
- DNA mutagen
- Endocrine (hormone) disruptive chemical (EDC)
Glyphosate Formulations:  
“Adjuvants”

Pesticide formulations contain “adjuvants” to allow “active ingredient” to work.

Adjuvants:

- Mostly chemically modified tallowamines (POEA)
- Claimed to be “inert”

Increasing evidence shows some adjuvant components more toxic than active ingredient:

Mesnage et al., 2012
### How much glyphosate in general human population?

**Table 1** Glyphosate concentrations in human urine samples (mean and maximum values) and resulting estimates of previous exposure, compared to ADI or AOEL

<table>
<thead>
<tr>
<th>References</th>
<th>Analytical method, LOD/LOQ</th>
<th>Participants</th>
<th>Urine concentrations [µg/l]</th>
<th>Estimated exposure or systemic dose [µg/kg bw]</th>
<th>Percentage of ADI or AOEL [%]</th>
</tr>
</thead>
</table>
| Acquavella et al. (2004)  | HPLC following ion exchange LOD 1 µg/L | 48 male farmers from Minnesota and South Carolina (USA), their spouses and 79 children | Mean: 3.2  
Maximum: 233 (farmer) 29 (child)* | Mean: 0.11 (systemic dose)  
Maximum: 8.3 (systemic dose) | 8.3 % of AOEL (maximum value), ca. 0.1 % of AOEL (mean value) |
| Curwin et al. (2007)      | Immunoassay (fluorescent microbeads) LOD 0.9 µg/L | 48 women, 47 men, 117 children from "farm" and "non-farm" households in Iowa | Mean: 1.1–2.7 (in different groups)  
Maximum: 18 (*farm child)" | Mean: 0.5 (dietary exposure highest mean)  
Maximum: 0.1 (systemic dose highest mean) | 0.1 % of ADI |
| Mesnagle et al. (2012)    | HPLC-MS LOD 1 µg/L LOQ 2 µg/L | 1 farmer, his wife and 3 children, presumably Europe | n.a. (only single values available)  
Maximum: 9.5 (farmer) 2 (child)* | Mean: 0.33 (systemic dose) | <0.4 % of AOEL |
| Hoppe (2013)              | GC-MS/MS following derivatisation LOQ 0.35 µg/L | 182 citizens from 18 European countries | Mean: 0.21  
Maximum: 1.82 | Mean: 0.3–0.4 (dietary exposure) | <0.1 % of ADI |
| Markard (2014)            | GC-MS/MS (presumably) LOD 0.15 µg/L | 40 male and female German students | n.a. (22 samples above LOQ)  
Maximum: 0.65 | Mean: 0.13 (dietary exposure) | <0.1 % of ADI |
| Krüger et al. (2014)      | ELISA partly validated against GC-MS LOD/LOQ not given | >300 (mostly from Germany) | Mean: ≤2  
Maximum: 5 | Mean: 0.83 (dietary exposure) | <0.2 % of ADI |
| Honeycutt and Rowlands   (2014) | ELISA LOQ 7.5 µg/L | 35 women, men and children from USA | n.a. (13 samples above LOQ)  
Maximum: 18.8 | Mean: 3.3 (dietary exposure)  
Maximum: 0.66 (systemic dose) | <0.7 % of ADI, <0.7 % of AOEL |

*n.a. not applicable*  
* For children, comparisons to reference values were not performed since age, body weight and urine volume were not known

Niemann et al., 2015
Levels detected to date in humans are below regulatory set limits but are they safe?

Need for long term laboratory animal toxicity feeding studies at ADI / cRfD or at real world exposure levels
Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize

Gilles-Eric Séralini¹*, Emilie Clair¹, Robin Mesnage¹, Steeve Gress¹, Nicolas Defarge¹, Manuela Malatesta², Didier Hennequin³ and Joël Spiroux de Vendômois¹

Only laboratory animal feeding study looking at long-term health effects of complete Roundup herbicide formulation
Study of toxic effects of Roundup herbicide in adult male and female rats under a daily regimen for 24 consecutive months

60 Rats
Roundup
(10 Males / 10 Females)
(3 doses)

Doses of Roundup (glyphosate equivalent):
half of agricultural dilution

400mg/L
50ng/L → 4ng/kg bw/day

75,000 times below EU glyphosate ADI
437,500 times below US glyphosate ADI
Summary of Séralini (2014) Study Findings on Lowest Dose Roundup Treatment Group

- 3 times more pathologies at anatomical level revealed by histological (microscopic) analysis
- Blood/urine biochemistry suggest impairment of liver and especially kidney function
- Testosterone (96% increase)/estrogen (26% decrease) imbalance suggesting endocrine disrupting effects
- Electron microscopy analysis of liver shows statistically significant alterations in cell nuclear structure suggesting major changes in gene function.
Molecular Profiling – “omics” analysis

- Comprehensive in-depth gene function and molecular composition analysis
- Highly sensitive
- Highly predictive or indicative of health or disease status

Transcriptomics - gene expression / function profile

Proteomics - protein content profile

Metabolomics - small molecule (metabolite) profile
Séralini et al. 2014: Two-year Animal Feeding Study Follow-up:

Oomics Results from the female animals administered with the lowest dose of Roundup (4ng/kg bw/day glyphosate)

Roundup residue consumption at a level permitted in drinking water (0.05 ppb glyphosate equivalent)
Transcriptome profile analysis of Roundup effects on the genes regulating liver and kidney function

The activity of 28,407 genes was measured by using the microarray technology (DNA chips)
Statistical analysis of the genes disturbed in female liver between R-treated (red spheres) and control (green spheres) rats.

Variations among the expression of 28,000 genes allow us to see the separation between individuals.

The Roundup treated samples clearly separate from the controls.
Results from Roundup treatment group have very high statistical significance

Each dot represents a gene; Common disturbances are in red

A « –log10 p-value » of 2 means that there is 1% chance that the disturbance is due to chance

A « –log10 p-value » of 4 is 0.01%

... 

Some genes have a « –log10 p-value » of 10, it means that there is 1 chance on 10,000,000,000 that the disturbance is due to chance

<table>
<thead>
<tr>
<th>p-value</th>
<th>Liver</th>
<th>Kidney</th>
<th>Random</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>8606(0.21)</td>
<td>8656(0.21)</td>
<td>1835(0.98)</td>
</tr>
<tr>
<td>0.01</td>
<td>4224(0.08)</td>
<td>4447(0.08)</td>
<td>380(0.96)</td>
</tr>
<tr>
<td>0.001</td>
<td>1593(0.02)</td>
<td>1894(0.02)</td>
<td>31(0.95)</td>
</tr>
<tr>
<td>0.0001</td>
<td>630(0.006)</td>
<td>764(0.005)</td>
<td>1(0.95)</td>
</tr>
<tr>
<td>0.00001</td>
<td>230(0.002)</td>
<td>219(0.002)</td>
<td>0</td>
</tr>
</tbody>
</table>
Many gene functions are disturbed in liver and kidneys by an ultra low dose of Roundup

As some gene disturbances can be due to secondary effects of the pathological status, we have chosen the most robust dataset for the study of primary mechanisms of Roundup toxicity.

1319 genes are commonly disturbed among liver and kidneys of females.

Statistical threshold recommended:
- $P < 0.01$
- Fold changes $> 1.1$

Livers: 2791, Kidneys: 3020, Overlapping 1319 genes.
Gene function disruptions explain the pathologies at the anatomical level

Organ damage
(Necrosis, fibrosis, ischemia, oxidative stress)
Transcriptome profiling reveals that long-term ultra-low ingestion of Roundup results in:

- Gene functional disturbances that predict liver and kidney structure and functional damage
- Can account for observed kidney and liver pathologies (necrosis and failure) seen at macroscopic (whole organ) and blood/urine biochemical levels in the Séralini study

Roundup ingestion well-below currently held safe regulatory levels is disruptive of liver and kidney structure and function.
Proteome and Metabolome profile analysis of Roundup effects on the liver

Multiomics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide

Robin Mesnage¹, George Renney², Gilles-Eric Séralini³, Malcolm Ward² & Michael N. Antoniou¹

Mensn R et al. (2017) Sci Rep, 7: 39328. DOI: 10.1038/srep39328
Long-term very low dose Roundup consumption results in increased plasma triglyceride fat levels in rats.

C, control;  R, Roundup treatment
Wide scale proteome and metabolome alteration in liver of female rats following long-term administration with an ultra-low dose of Roundup
Proteome alteration reflects a liver lipotoxic condition

214 out of 1906 detected proteins significantly altered

- Peroxisomal proliferation, induction_liver
- Steatosis, development_liver
- Apoptosis via Mitochondrial membrane dysfunction
- Progression of oxidative stress
- Ischemia-induced cellular changes_liver

-log FDR adjusted p-values
Metabolome alteration reveals oxidative stress and lipotoxic state of liver in female rats administered with 673 metabolites detected. 55 significantly altered confirm lipotoxic and oxidative stress (glutathione and ascorbate). Alterations are markers of liver toxicity (γ-glutamyl dipeptides, acylcarnitines, and derivatives of proline).
Rat Liver Proteome and Metabolome

Conclusions

Metabolome and proteome disturbances show that Roundup at a dose thousands of times below regulatory permitted levels of daily ingestion reveal:

- Substantial overlap with hallmarks (biomarkers) of non-alcoholic fatty liver disease (NAFLD) and its progression to severe fatty liver disease - non-alcoholic steatohepatitis (NASH)

- Liver functional dysfunction resulting from chronic ultra-low dose glyphosate-based herbicide (Roundup) exposure
NAFLD and NASH: The New Plague of the 21st Century?

• **NAFLD**: Affects 25% of US citizens and 20-30% of Europeans
• **NAFLD** (5-10% of liver replaced by fat); ‘mild’ symptoms: fatigue, weakness, weight loss, loss of appetite, nausea, abdominal pain, spider-like blood vessels, yellowing of the skin and eyes (jaundice), itching, fluid build-up and swelling of the legs (edema) and abdomen (ascites), mental confusion.
• **NASH**: liver swelling; damage; cirrhosis.
• **Heightened risk of liver cancer, hence these findings relevant to current controversy of glyphosate link with cancer.**
• **Note**: liver cancer rates up 3% per year since 2000.
• **Risk factors**: overweight or obese, diabetes, high cholesterol or high triglycerides in the blood; rapid weight loss and poor eating habits.
• **NOTE**: some people develop NAFLD even if they do not have any risk factors
• **Is GBH exposure a new risk factor?**
Human exposures to GBHs are rising

Glyphosate is now authoritatively classified as a probable human carcinogen

Regulatory estimates of tolerable daily intakes for glyphosate in the United States and European Union are based on outdated science

Safe daily intake of glyphosate currently unknown
Industry study: rats fed commercialised Roundup tolerant GM NK603 corn for 90 days

Signs of toxic effects on liver and kidney function. (Hammond et al., 2004; see de Vendomois et al., 2009).

Academic studies: mice fed commercialised Roundup tolerant GM soya

- **Mice fed GM soy**: disturbed liver, pancreas and testes function (Malatesta et al., 2002; Malatesta et al., 2003; Vecchio et al., 2004).

- **Mice fed GM soy over 24 months**: more acute signs of ageing in the liver (Malatesta et al., 2008):

  Is GM process or Roundup residues or both in feed causing these effects?
Health effects of GMOs can be linked to pesticide residues

Ultrastructure of the hepatocytes of mice fed with a GM Roundup tolerant soy is typically desorganized (b) in comparison to control (a) (Malatesta et al., 2002)

The same profile is obtained by treating hepatocytes directly with Roundup herbicide
- World’s most used pesticide: 100-fold increased use since 1974; 2/3rd of total use in last 10 years (Benbrook, 2016)
- 80% GM crops are tolerant to glyphosate
- Majority of GM soy

### GM soy (USA):
- Glyphosate: average 3.3mg/kg
- AMPA: average 5.7 mg/kg

[Bohn et al., 2014]
Basis of Claims for Glyphosate Safety

Glyphosate safety due to perceived:

- Low toxicity based solely on EPSPS enzyme inhibition (shikimate biochemical pathway); present in plants & some bacteria/fungi only

- Rapid excretion via urine / faeces
  No bioaccumulation

- [Assumption: urine levels will always be higher than those in blood, breast milk, organs]
Limited toxicokinetics studies for glyphosate, but ..... 

Single very high dose analysis in rats shows:

- Ready uptake from the gastrointestinal tract from aqueous solutions (23-40%)
- Easy diffusion into tissues
- Conversion to AMPA

[Brewster et al., 1991; Anadon et al., 2009]

However note:
- Impossible to determine bioaccumulation from single dose exposures
- No ADI / cRfD tested, especially on long term basis
- No long-term testing of complete Roundup formulation
Glyphosate EDC Effects

Endocrine disruptor (can have effects at extremely low exposure levels over short or prolonged time):

- **Retinoic acid interference** (Paganelli A et al., 2010):
  - Birth defects

- **Estrogen interference** (Lin and Garry, 2000; Hokanson R et al., 2007; Thongprakaisang S et al., 2013):
  - Mimics estrogen action; augments estrogen function
  - **Organ damage; tumour growth promotion** (e.g. breast cancer)