Glyphosate: Harmless Tool or Sneaky Poison?
EU Parliament, May 10, 2017

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?***

Environmental & Analytical
Toxicology

Antoniou et al., J Environ Anal Toxicol 2012, S:4
http://dx.doi.org/10.4172/2161-0525.S4-006

Review Article Open Access

Teratogenic Effects of Glyphosate-Based Herbicides: Divergence of Regulatory Decisions from Scientific Evidence

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[8]*Director, Earth Open Source. Interests: employed at a GMO testing and certification company*

Abstract

The publication of a study in 2010, showing that a glyphosate herbicide formulation and glyphosate alone caused malformations in the embryos of *Xenopus laevis* and chickens through disruption of the retinoic acid signalling pathway, caused scientific and regulatory controversy. Debate centred on the effects of the production and consumption of genetically modified Roundup Ready® soy, which is engineered to tolerate applications of glyphosate herbicide. The study, along with others indicating teratogenic and reproductive effects from glyphosate herbicide exposure, was rebutted by the German Federal Office for Consumer Protection and Food Safety, BVL, as well as in industry-sponsored papers. These rebuttals relied partly on unpublished industry-sponsored studies commissioned for regulatory purposes, which, it was claimed, showed that glyphosate is not a teratogen or reproductive toxin.

However, examination of the German authorities' draft assessment report on the industry studies, which underlies glyphosate's EU authorisation, revealed further evidence of glyphosate's teratogenicity. Many of the malformations found were of the type defined in the scientific literature as associated with retinoic acid teratogenesis. Nevertheless, the German and EU authorities minimized these findings in their assessment and set a potentially unsafe acceptable daily intake (ADI) level for glyphosate. This paper reviews the evidence on the teratogenicity and reproductive toxicity of glyphosate herbicides and concludes that a new and transparent risk assessment needs to be conducted. The new risk assessment must take into account all the data on the toxicity of glyphosate and its commercial formulations, including data generated by independent scientists and published in the peer-reviewed scientific literature, as well as the industry-sponsored studies.

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

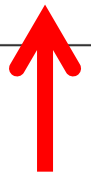
How much glyphosate in general human population?

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

References	Analytical method, LOD/LOQ	Participants	Urine concentrations [µg/l]		Estimated exposure or systemic dose [µg/kg bw]		Percentage of ADI or AOEL [%]
			Mean	Maximum	Mean	Maximum	
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	**3.2**	**233** (farmer) **29** (child)*	**0.11** (systemic dose)	**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	**1.1–2.7** (in different groups)	**18** ("farm child")*	**0.5** (dietary exposure highest mean) **0.1** (systemic dose highest mean)		**0.1** % of ADI
Mesnage 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)	**9.5** (farmer) **2** (child)*	**0.33** (systemic dose)		<**0.4** % of AOEL
Hoppe (2013)	GC–MS/MS following derivatisation LOQ 0.15 µg/L	**182** citizens from 18 European countries	**0.21**	**1.82**		**0.3–0.4** (dietary exposure)	<**0.1** % of ADI
Markard (2014)	GC–MS/MS (presumably) LOQ 0.15 µg/L	**40** male and female German students	**n.a.** (22 samples above LOQ)	**0.65**		**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)	≤**2**	**5**		**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)	**18.8**		**3.3** (dietary exposure) **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

Séralini *et al. Environmental Sciences Europe* 2014, **26**:14
http://www.enveurope.com/content/26/1/14

Environmental Sciences Europe
a SpringerOpen Journal

RESEARCH Open Access

Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize

Gilles-Eric Séralini[1*], Emilie Clair[1], Robin Mesnage[1], Steeve Gress[1], Nicolas Defarge[1], Manuela Malatesta[2], Didier Hennequin[3] and Joël Spiroux de Vendômois[1]

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% decease) 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:

Omics 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

Mesnage *et al. Environmental Health* (2015) 14:70
DOI 10.1186/s12940-015-0056-1

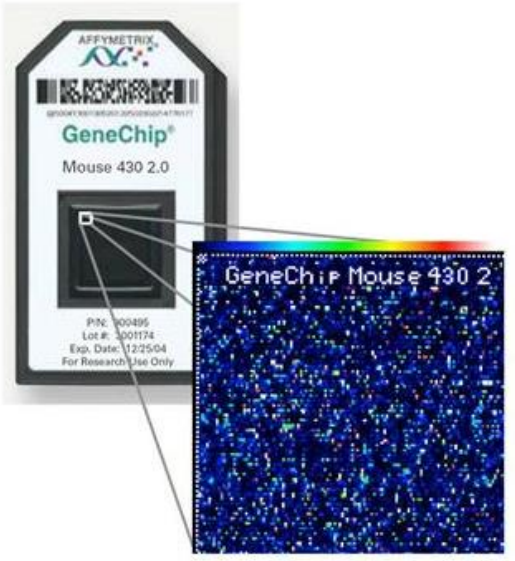
ENVIRONMENTAL HEALTH

RESEARCH Open Access

Transcriptome profile analysis reflects rat liver and kidney damage following chronic ultra-low dose Roundup exposure

Robin Mesnage[1], Matthew Arno[2], Manuela Costanzo[3], Manuela Malatesta[3], Gilles-Eric Séralini[4] and Michael N. Antoniou[1*]

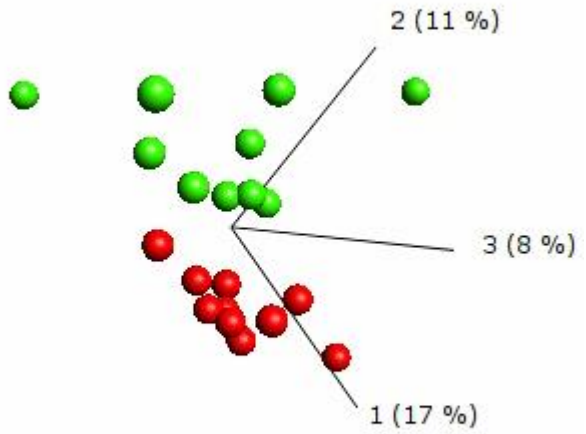
Mesnage et al., 2015

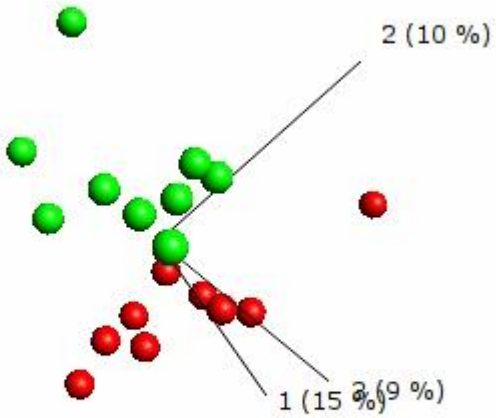


The activity of 28,407 genes was measured by using the microarray technology (DNA chips)

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.

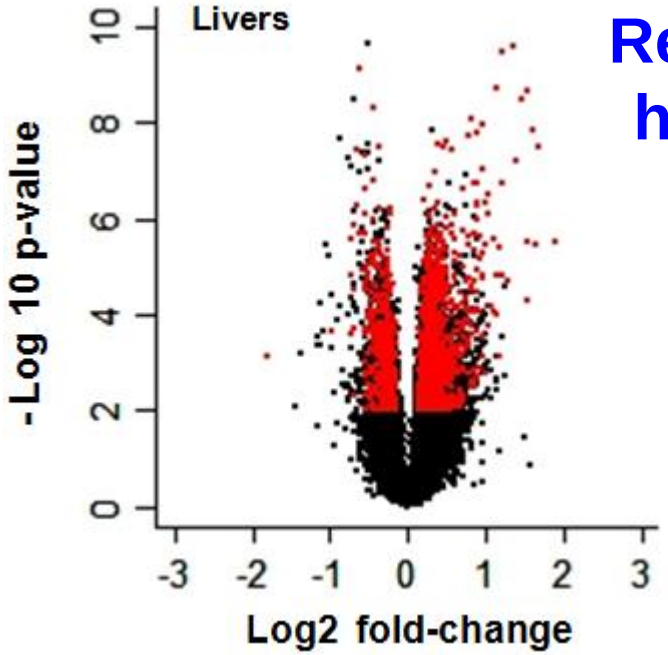


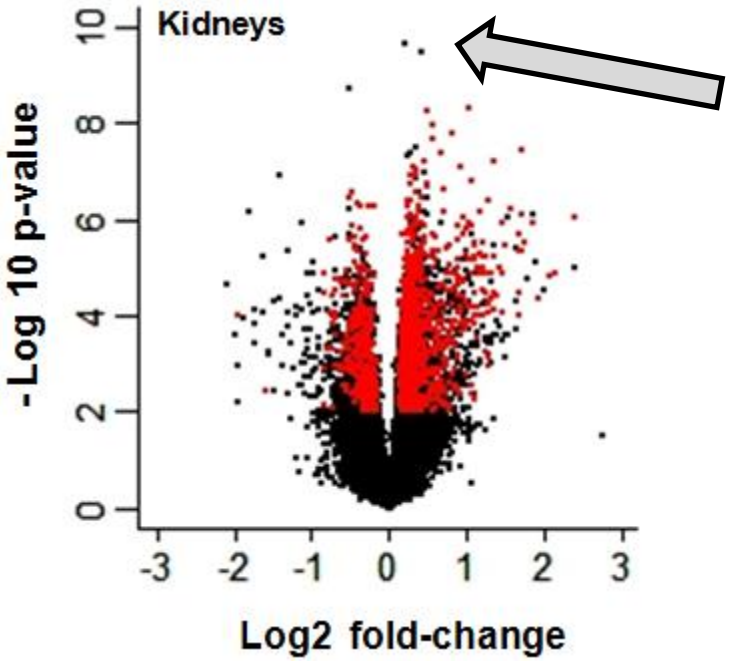


FEMALE LIVERS

FEMALE KIDNEYS

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%

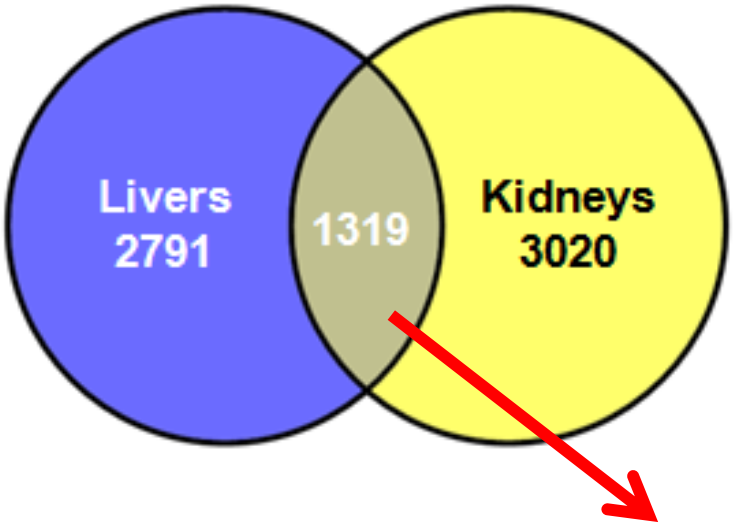
…

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

p-value	Liver	Kidney	Random
0.05	8606$^{(0.21)}$	8656$^{(0.21)}$	1835$^{(0.98)}$
0.01	4224$^{(0.08)}$	4447$^{(0.08)}$	380 $^{(0.96)}$
0.001	1593$^{(0.02)}$	1894$^{(0.02)}$	31$^{(0.95)}$
0.0001	630$^{(0.006)}$	764$^{(0.005)}$	1$^{(0.95)}$
0.00001	230$^{(0.002)}$	219$^{(0.002)}$	0

Many gene functions are disturbed in liver and kidneys by an ultra low dose of Roundup

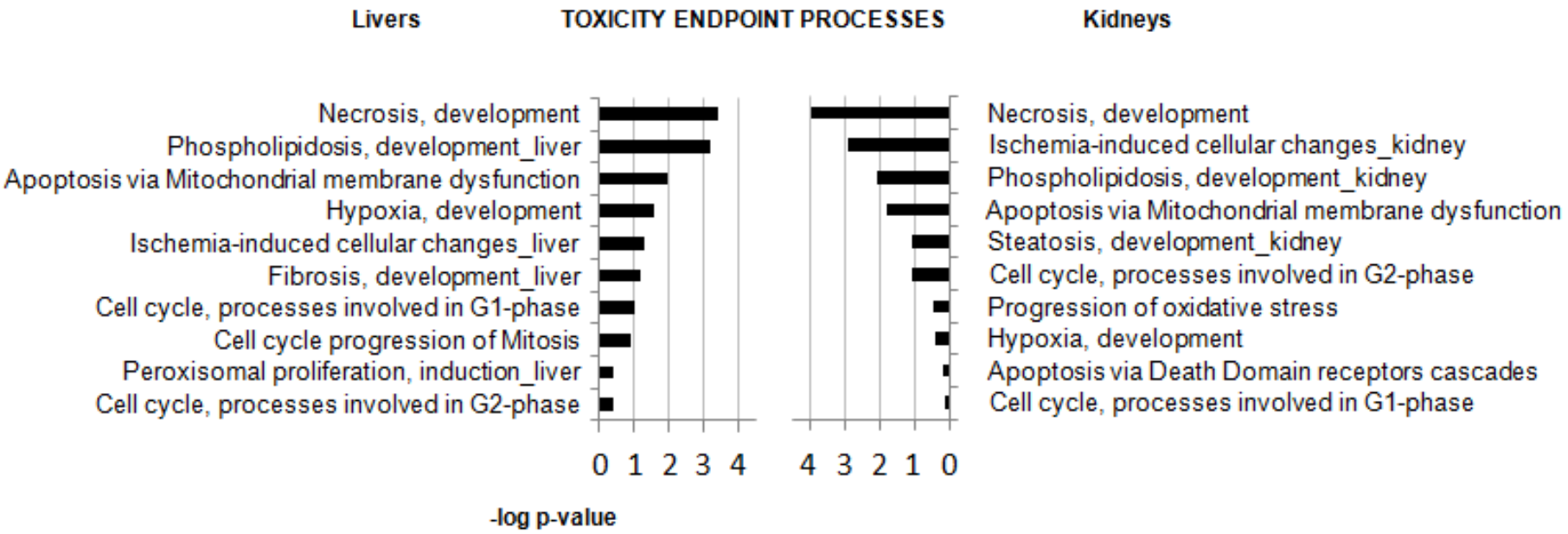
As some gene disturbances can be due to secondary effects of the pathological status,
we have chosen the most robust dataset for the study of primary mechanisms of Roundup toxicity



***Statistical threshold recommended**:*
P < 0.01
Fold changes > 1.1

1319 genes are commonly disturbed among liver and kidneys of females

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

SCIENTIFIC REPORTS

OPEN

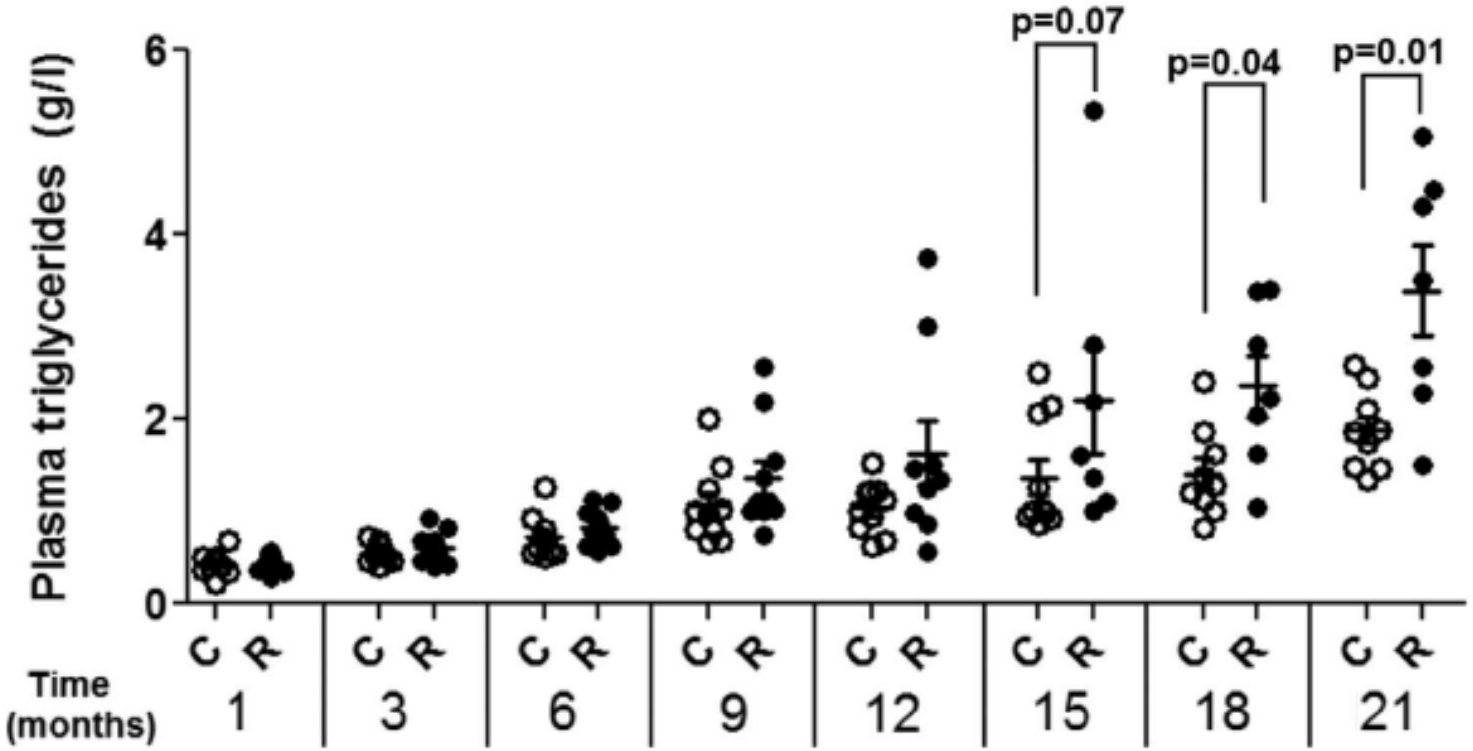
Multiomics reveal non-alcoholic fatty liver disease in rats following chronic exposure to an ultra-low dose of Roundup herbicide

Received: 22 July 2016
Accepted: 22 November 2016
Published: 09 January 2017

Robin Mesnage[1], George Renney[2], Gilles-Eric Séralini[3], Malcolm Ward[2] & Michael N. Antoniou[1]

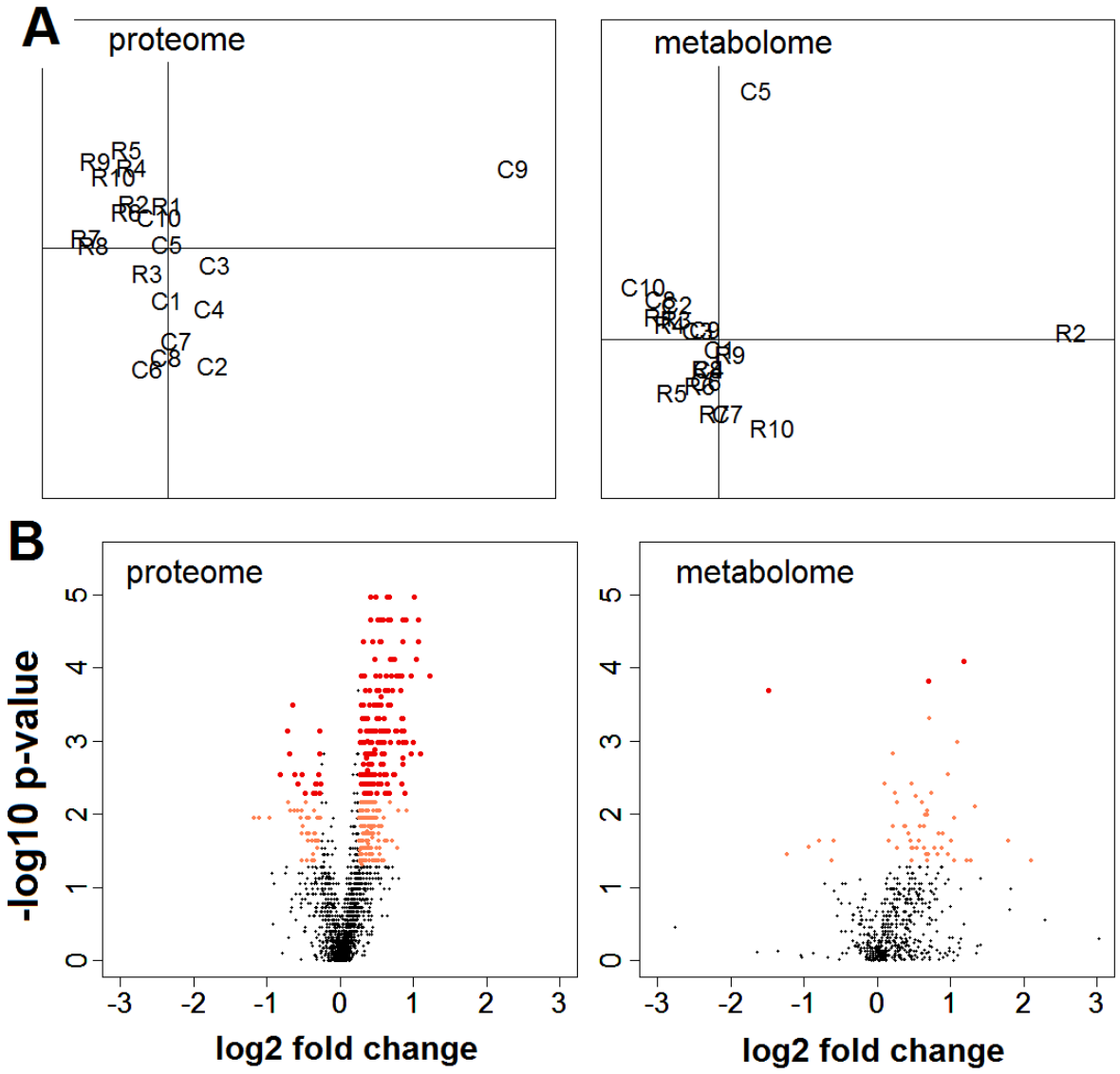
Mesnage R et al. (2017) *Sci Rep*, **7**: 39328. DOI: 10.1038/srep39328

Long-term very low dose Roundup consumption results in increased plasma triglyceride fat levels in rats



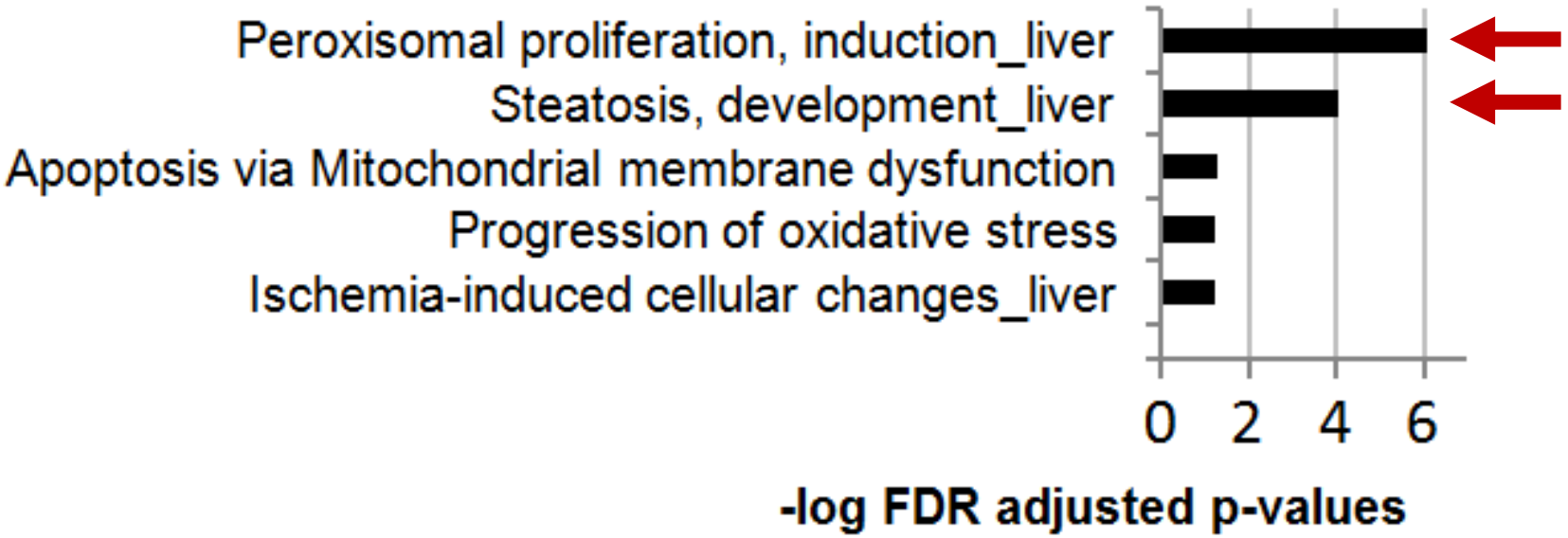
C, control; **R, Roundup treatment**

Wide scale proteome and metabolome alteration in liver of female rats following long-term administration with an ultra-low dose of Roundup

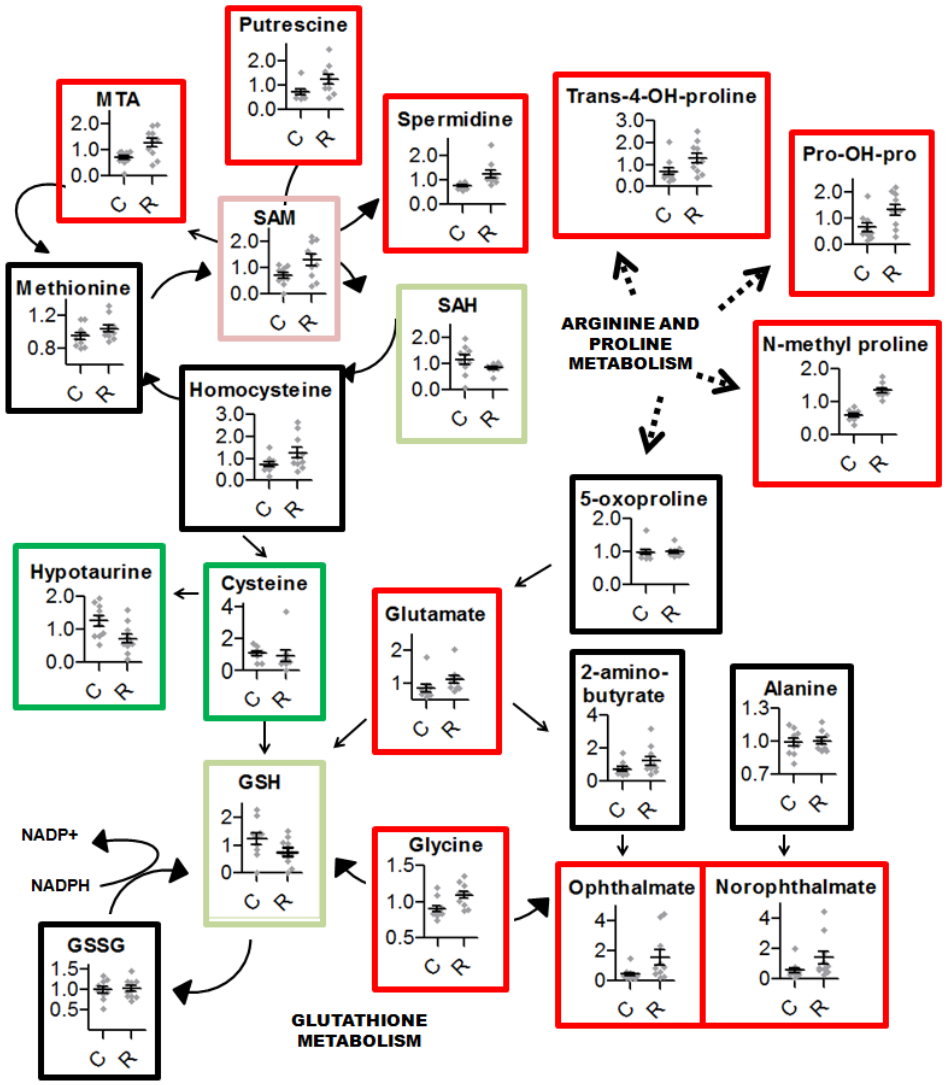


Proteome alteration reflects a liver lipotoxic condition

214 out of 1906 detected proteins significantly altered



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 steatohepatosis (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?**

Myers et al. Environmental Health (2016) 15:19
DOI 10.1186/s12940-016-0117-0

Environmental Health

REVIEW Open Access

Concerns over use of glyphosate-based herbicides and risks associated with exposures: a consensus statement

John Peterson Myers[1,13*], Michael N. Antoniou[2], Bruce Blumberg[3], Lynn Carroll[4], Theo Colborn[4], Lorne G. Everett[5], Michael Hansen[6], Philip J. Landrigan[7], Bruce P. Lanphear[8], Robin Mesnage[2], Laura N. Vandenberg[9], Frederick S. vom Saal[10], Wade V. Welshons[11] and Charles M. Benbrook[12*]

- **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).

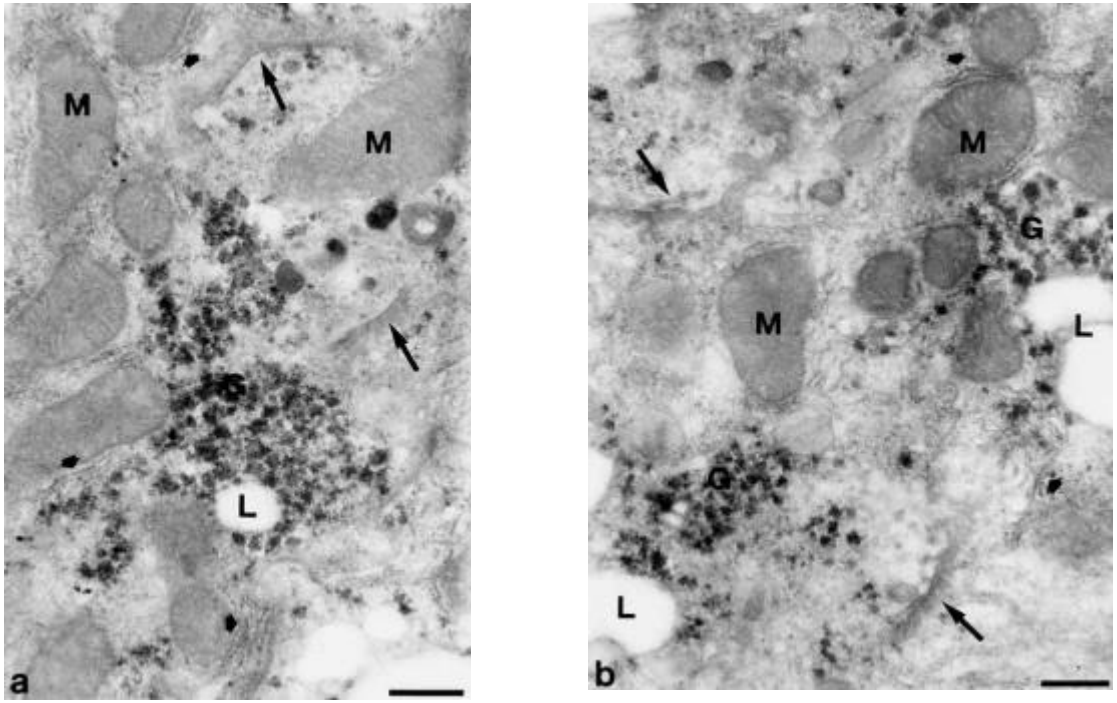
Academic studies: mice fed commercialised Roundup tolerant GM soya

- **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?

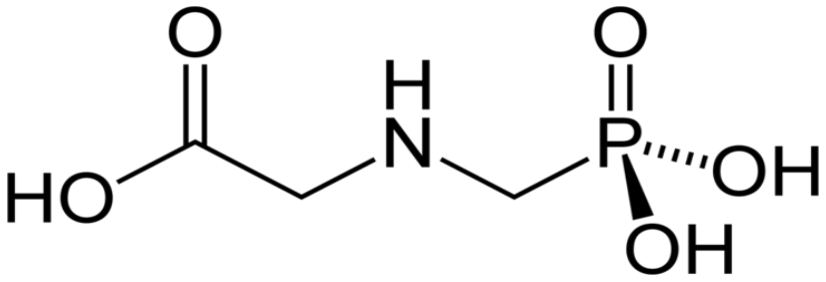
Health effects of GMOs can be linked to pesticide residues



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

Glyphosate



- **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)**