HEAL Webinar – June 28th 2021

Health impact of glyphosate and glyphosate-based herbicides

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY STUDY USING SPRAGUE-DAWLEY RATS EXPOSED UNDER VARIOUS CALENDARS TO THE WEED-KILLER GLYPHOSATE AND A COMMERCIAL FORMULATION GLYPHOSATE-BASED

FIORELLA BELPOGGI, SCIENTIFIC DIRECTOR
RAMAZZINI INSTITUTE, BOLOGNA –ITALY

Istituto Ramazzini
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The Global Glyphosate study

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Questions

1. *"Have glyphosate and GBHs been tested at currently acceptable daily intake and real-world levels of exposure?"*
2. *"Does glyphosate and its formulations have different effects?"*
3. *"What are the possible adverse effects of GBHs other than cancer?"*

Answer: The Global Glyphosate Study, an integrated GLP in vivo study

➔ In 2016 the Ramazzini Institute started the Pilot Phase (STAGE I) of the Global Glyphosate Study (STAGE II), involving multiple independent Institutions and Universities in Europe and the U.S.

Partners

- **University of Bologna (Dpt. of Agriculture; Dpt. Veterinary Medicine; Dpt. of Economics, Management and Statistics), Italy**
- **King's College London, Gene Expression and Therapy Group, Dept. of Medical and Molecular Genetics, London, UK**
- **Istituto Superiore Sanità, Dept. of Food Safety and Veterinary Public Health Rome, Italy**
- **Environmental Carcinogenesis Unit, Ospedale Policlinico San Martino, Genova, Italy**
- **Mount Sinai School of Medicine, New York, NY, USA**
- **George Washington University, Washington, DC, USA**

STAGE I

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THE RAMAZZINI INSTITUTE 13-WEEK STUDY ON GLYPHOSATE-BASED HERBICIDES AT HUMAN EQUIVALENT EXPOSURE LEVELS IN SPRAGUE-DAWLEY RATS

AIMS:

- To assess the methodology for conducting a GLP long-term integrated and comprehensive study (ongoing)

- To investigate whether exposure to Glyphosate or Roundup affects the development and endocrine system across different life stages of treated Sprague-Dawley rats at human equivalent US Acceptable Daily Intake (1.75mg/Kg bw)

STAGE I: experimental plan

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GROUP	TREATMENT	DOSE
I	Drinking water (DW)	-
II	DW + Glyphosate	USA ADI (1.75 mg/Kg bw/day)
III	DW + Roundup	USA ADI (1.75 mg/Kg bw/day) Glyphosate equivalent

- **Route of Administration:** *ad libitum* in drinking water
- **Test Substances:** Glyphosate (purity > 99.5%) Roundup Bioflow (MON 52276)

STAGE I: experimental design

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Glyphosate or Roundup (MON 52276)
(1.75 mg/Kg/bw = cRfD USA)

Gestation and lactation

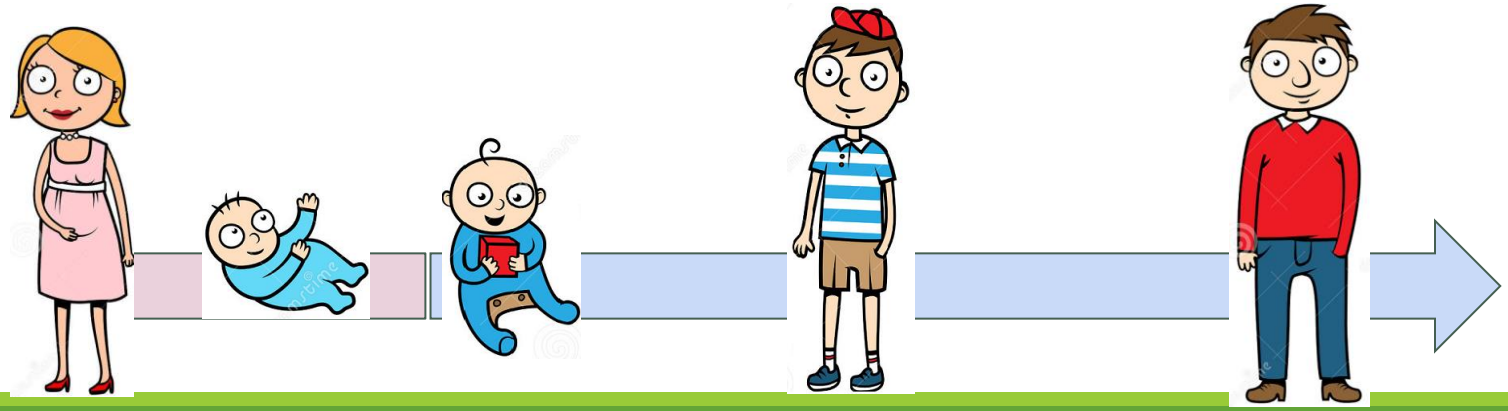
(8 dams/group)

Pre-puberty **Puberty**

6-week cohort (8/sex/group) **PND 70**

Pre-puberty **Puberty** **Adulthood**

13-week cohort (10/sex/group) **PND 120**



GBHs: Key characteristics of endocrine disruptors

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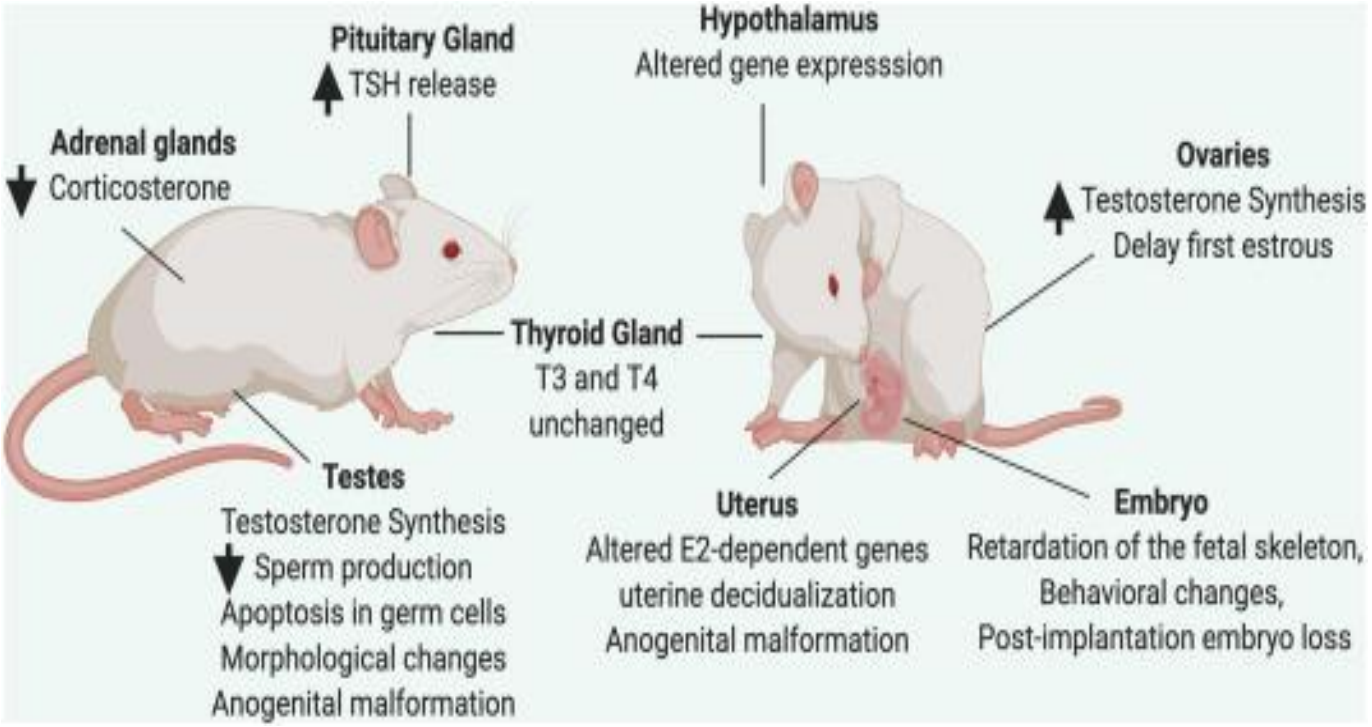
Evidences in *in vivo* model



Munoz JP, *et al* Glyphosate and the key characteristics of an endocrine disruptor: A review, Chemosphere, 2020 (in press)

STAGE I: endpoints evaluated

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Endocrine sensitive endpoints

Group		Body weight	Water and feed cons.	Urinanalysis	Clinical chemistry	Haematological tests	Organ weight	Histopathology	Micronuclei	Transcriptome	Microbiome	Litter size	Live birth index	Sex ratio	Intra/extra uterine death	Anogenital distance	Balano-preputial sep.	Vaginal opening	First estrous	Estrous cyclicity	Hormone analyses	Sperm analyses	Sperm aneuploidy
I (control)	F0	√	√				√	√	√		√										√		
	F1	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
II (Glyphosate)	F0	√	√				√	√	√		√				√						√		
	F1	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√
III (Roundup)	F0	√	√				√	√	√		√				√						√		
	F1	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√	√

STAGE I: in life endocrine - sensitive endpoints

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Start of treatment

Weaning

GD 6

PND 28

Maternal reproductive outcome of **dams**

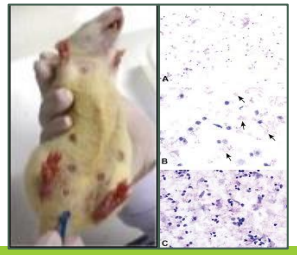
6-week cohort

13-week cohort

Anogenital distance (PND 4)
Litter data (sex ratio, size, survival index, …)

PND 35

PND 95



First estrus

Estrous cycle

STAGE I: end of life endpoints

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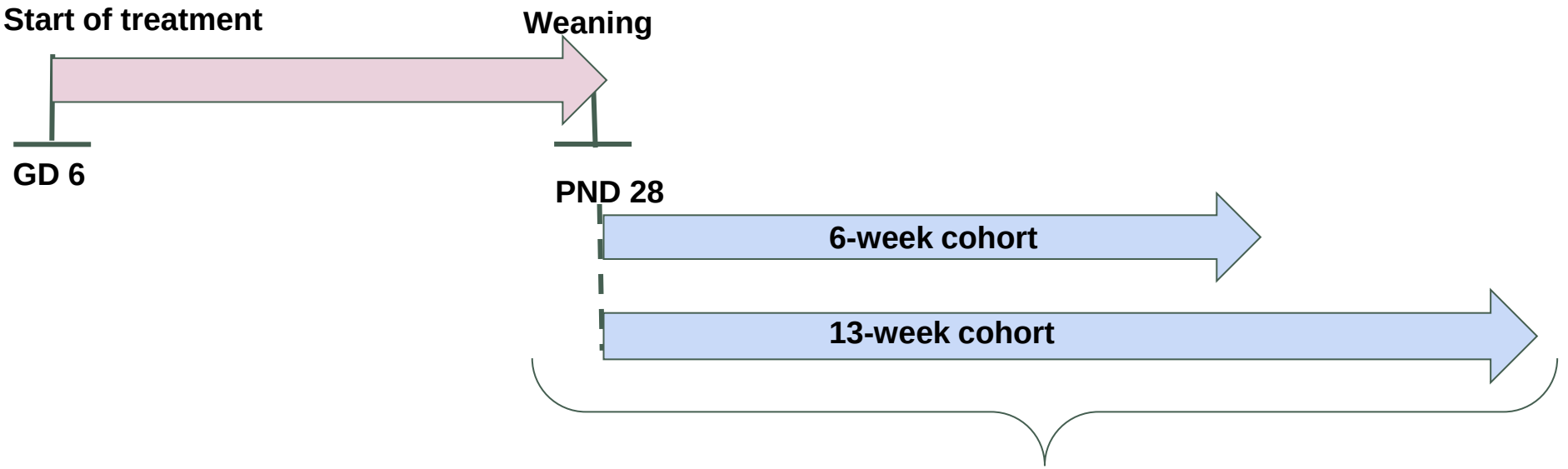
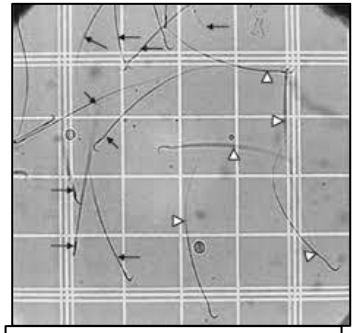
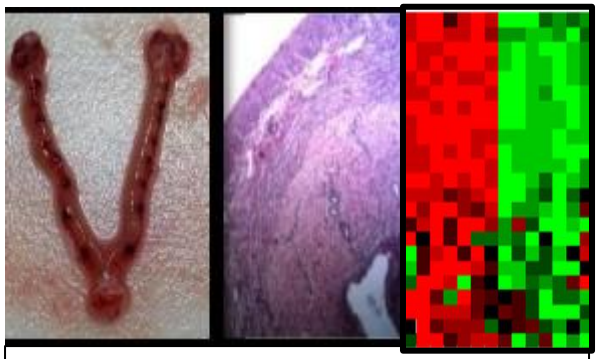


Hormone and haematological analysis; clinical chemistry



Sperm analysis



Histopathology/Transcriptome

STAGE I: results

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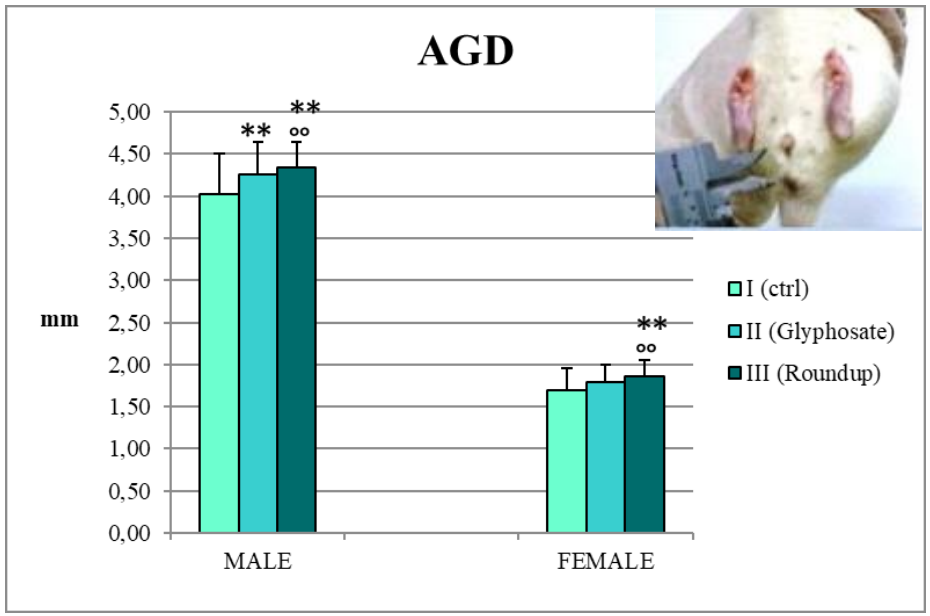
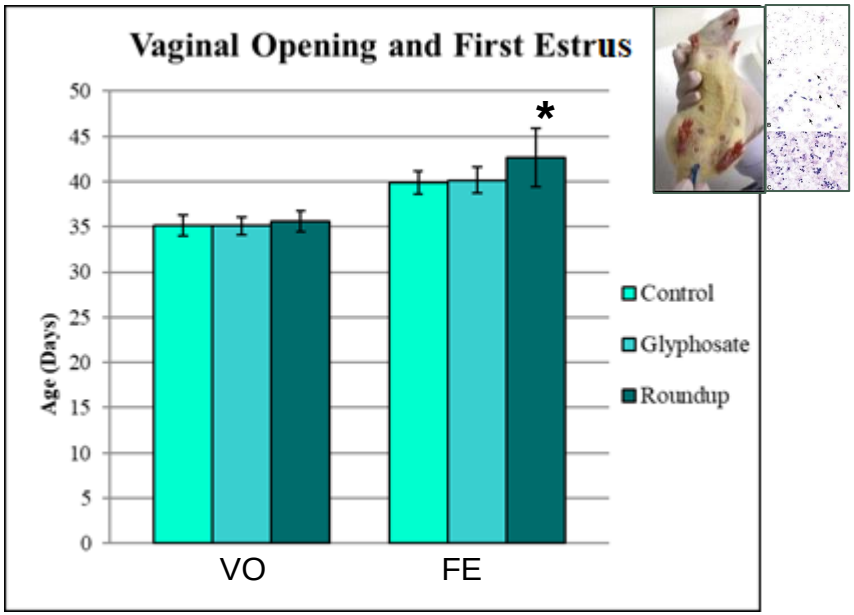


Analysis performed considering the body weight at evaluation of AGD
**: p<0.01 with multilevel linear regression
°° : p<0.01 with multilevel linear regression with litter as random effect



*: p< 0.05

- In pups, **anogenital distance (AGD) at PND 4 was significantly increased** both in Roundup-treated males and females and in glyphosate-treated males
- **Age at first estrus (FE) was significantly delayed in the Roundup** exposed group

STAGE I: results:_hormones in females

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Serum Hormones	6-week cohort			13-week cohort		
	Control	Glyphosate	Roundup	Control	Glyphosate	Roundup
No. of females examined	8 (8)	8 (8)	8 (8)	10 (10)	10 (10)	10 (10)
TT (ng/ml)	0.66 ± 0.064	0.75 ± 0.12	0.68 ± 0.11 [a]	0.51 ± 0.06	0.72 ± 0.10	**0.72 ± 0.07 [b] ***
fT (pg/ml)	6.49 ± 1.00 [c]	6.74 ± 1.89 [d]	7.70 ± 1.35 [a]	9.18 ± 2.49	12.04 ± 1.25	12.52 ± 1.76 [b]
DHT (pg/ml)	294.28 ± 50.40	328.34 ± 51.93 [a]	488.94 ± 114.68 [a]	382.93 ± 52.14	460.09 ±60.06	268.84 ± 45.56 [b]
SHBG (ng/ml)	864.82 ± 30.24	952.75 ± 54.98	903.07 ± 29.61	968.27 ± 21.39	993.44 ± 32.79	964.81 ± 27.20
E2 (pg/ml)	14.95 ± 7.24	32.24 ± 8.77	66.96 ± 25.17	18.08 ± 8.49	28.48 ± 13.71	43.91 ± 9.92
Plasma Hormones	**6-week cohort**			13-week cohort		
	Control	Glyphosate	Roundup	Control	Glyphosate	Roundup
No. of females examined	7 (8)	7 (8)	6 (8)	7(10)	5(10)	6(10)
FSH (ng/ml)	3.95 ± 2.50	2.67 ± 1.22	3.15 ± 1.65	1.58 ± 0.51	1.73 ± 0.64	1.46 ± 0.35
LH (ng/ml)	5.75 ± 3.04	4.86 ± 1.93	4.52 ± 3.38	1.83 ± 0.25	2.35 ± 1.11	2.16 ± 1.28
PRL (ng/ml)	102.34 ± 164.71 [c]	27.49 ± 30.23	46.49 ± 31.03	-	-	-
GH (ng/ml)	12.61 ± 13.30	3.85 ± 0.97 [c]	4.16 ± 2.84	-	-	-
TSH (ng/ml)	2.70 ± 1.13	3.02 ± 2.00	3.04 ± 1.53	-1.29 ± 0.69 [e]	-1.93 ± 0.89 [f]	-3.03 ± 2.22 [e]
ACTH (pg/ml)	331.60 ± 89.59	314.09 ± 170.60	354.95 ± 104.96	-	-	-
BDNF (pg/ml)	245.03 ± 155.68	483.62 ± 301.02	351.33 ± 177.28	-25399 ± 155.77 [e]	-377.79 ± 226.30 [f]	-249.39 ± 14566 [e]

[a]: 7 out 8; [b]: 9 out 10; [c]: 6 out 8; [d]: 5 out 8; [e]: 4 out 10; [f]: 2 out 10

[g] : Not statistically evaluated due to insufficient sample size after clustering on the basis of the estrous cycle

* $p < 0.05$ with Kruskal-Wallis' tests ** $p < 0.01$ with Kruskal-Wallis' tests note: data reported as Mean ± standard deviation

STAGE I: results_hormones in males

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Serum Hormones	6-week cohort			13-week cohort		
	Control	Glyphosate	Roundup	Control	Glyphosate	Roundup
No. of males examined	8 (8)	8 (8)	8 (8)	10 (10)	10 (10)	10 (10)
TT (ng/ml)	1.12 ± 0.12	1.02 ± 0.28	0.84 ± 0.11 [a]	8.16 ± 2.86	7.65 ± 2.86	3.76 ± 0.90
fT (pg/ml)	14.53 ± 2.37	7.45 ± 2.23 [b]	13.12 ± 3.74 [b]	296.70 ± 123.70 [c]	724.24 ± 419.22 [c]	90.40 ± 29.44
DHT (pg/ml)	761.11 ± 136.21	575.28 ± 238.24	554.29 ± 145.16 [a]	15709.0 ± 5547.20	16711.8 ± 6724.5	**1980.2 ± 664.68[c]**** ↓
SHBG (ng/ml)	861.20 ± 30.24	833.24 ± 21.15	856.78 ± 32.39	917.58 ± 16.94	906.36 ± 21.62	906.51 ± 18.89
E2 (pg/ml)	1.04 ± 0.21[a]	3.29 ± 1.85	6.19 ± 2.28 [b]	3.66 ± 2.57 [c]	1.08 ± 0.02[d]	6.00 ± 1.11
Plasma Hormones	**6-week cohort**			**13-week cohort**		
	Control	**Glyphosate**	**Roundup**	**Control**	**Glyphosate**	**Roundup**
No. of males examined	7 (8)	6 (8)	7 (8)	10 (10)	10 (10)	10 (10)
FSH (ng/ml)	7.00 ± 1.38	6.43 ± 1.16	7.18 ± 0.68	2.32 ± 0.40 [e]	2.18 ± 0.16 [f]	2.90± 0.28 [f]
LH (ng/ml)	3.76 ± 0.79	2.87 ± 0.63	4.41 ± 0.62	1.20 ± 0.17 [e]	1.25 ± 0.24 [d]	1.40 ± 0.18 [f]
PRL (ng/ml)	3.83 ± 0.64	3.00 ± 0.64	4.31 ± 1.32	-	-	-
GH (ng/ml)	6.03 ± 4.32 [g]	23.19 ± 21.17 [h]	4.38 ± 1.94 [i]	-	-	-
TSH (ng/ml)	4.23 ± 0.76	**8.17 ± 1.58*** ↑	5.57 ± 0.31 [b]	1.89 ± 0.20	2.53 ± 0.25	**3.69 ± 0.42 **** ↑
ACTH (pg/ml)	346.67 ± 35.52	255.18 ± 43.29	292.26 ± 26.22	-	-	-
BDNF (pg/ml)	99.49 ± 25.32 [b]	148.85 ± 37.53	**171.79 ± 14.65*** ↑	53.83 ± 14.77 [c]	58.07 ± 13.83	45.15 ± 14.64

[a]: 7 out 8; [b]: 6 out 8; [c]: 9 out 10; [d]: 8 out 10; [e]: 6 out 10; [f]: 7 out 10; [g]: 5 out 8; [h]: 3 out 8; [i]: 4 out 8

* $p < 0.05$ with Kruskal-Wallis' tests ** $p < 0.01$ with Kruskal-Wallis' tests note: data reported as Mean ± standard deviation

STAGE I: other important results

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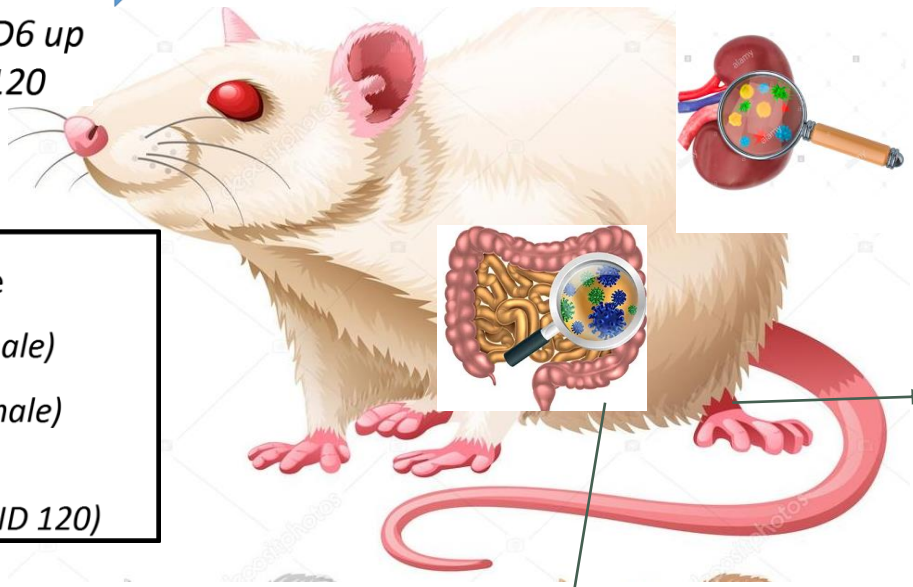
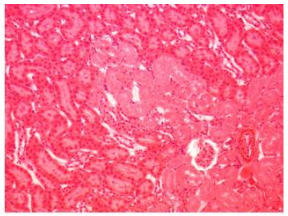
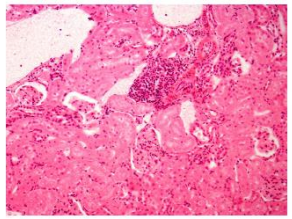
Glyphosate or Roundup (MON 52276)
(1.75 mg/Kg/bw = cRfD USA)

X8 per group from GD6 up to PND70 or PND 120







Renal tubular degeneration *(only dams-A)*

Focal minimal inflammation *(only dams-B)*

(only Roundup)

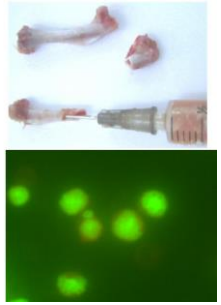
↑ Inorganic Phosphate

↓ Creatinine *(only female)*

↓ Total protein *(only male)*

↑ Lymphocyte Count
(only Roundup at PND 120)

MN-PCE ‰
(only Roundup at PND 70)



↑ *Bacteriodetes (Prevotella)* ↓ *Firmicutes (Lactobacillus)*

(both Glyphosate and Roundup at PND 31)

STAGE I: publications

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Panzacchi et al. Environmental Health (2018) 17:52
https://doi.org/10.1186/s12940-018-0393-y

Environmental Health

RESEARCH Open Access

The Ramazzini Institute 13-week study on glyphosate-based herbicides at human-equivalent dose in Sprague Dawley rats: study design and first in-life endpoints evaluation

Simona Panzacchi[1†], Daniele Mandrioli[1,2†], Fabiana Manservisi[1,3], Luciano Bua[1], Laura Falcioni[1], Marcella Spinaci[3], Giovanna Galeati[3], Giovanni Dinelli[2], Rossella Miglio[4], Alberto Mantovani[5], Stefano Lorenzetti[5], Jianzhong Hu[6], Jia Chen[7], Melissa J. Perry[8], Philip J. Landrigan[9] and Fiorella Belpoggi[1*]

Manservisi et al. Environmental Health (2019) 18:15
https://doi.org/10.1186/s12940-019-0453-y

Environmental Health

RESEARCH Open Access

The Ramazzini Institute 13-week pilot study glyphosate-based herbicides administered at human-equivalent dose to Sprague Dawley rats: effects on development and endocrine system

Fabiana Manservisi[1,2†], Corina Lesseur[3†], Simona Panzacchi[1], Daniele Mandrioli[1,4], Laura Falcioni[1], Luciano Bua[1], Marco Manservigi[1], Marcella Spinaci[2], Giovanna Galeati[2], Alberto Mantovani[5], Stefano Lorenzetti[5], Rossella Miglio[6], Anderson Martino Andrade[7], David Møbjerg Kristensen[8], Melissa J. Perry[9], Shanna H. Swan[3], Jia Chen[3] and Fiorella Belpoggi[1*]

Mao et al. Environmental Health (2018) 17:50
https://doi.org/10.1186/s12940-018-0394-x

Environmental Health

RESEARCH Open Access

The Ramazzini Institute 13-week pilot study on glyphosate and Roundup administered at human-equivalent dose to Sprague Dawley rats: effects on the microbiome

Qixing Mao[1,2†], Fabiana Manservisi[3,4†], Simona Panzacchi[3], Daniele Mandrioli[3,5], Ilaria Menghetti[3], Andrea Vornoli[3], Luciano Bua[3], Laura Falcioni[3], Corina Lesseur[6], Jia Chen[6], Fiorella Belpoggi[3*] and Jianzhong Hu[1]

scientific reports

OPEN

Low-dose exposure of glyphosate-based herbicides disrupt the urine metabolome and its interaction with gut microbiota

Jianzhong Hu[1,2*], Corina Lesseur[2], Yu Miao[2], Fabiana Manservisi[3,4], Simona Panzacchi[3], Daniele Mandrioli[3,5], Fiorella Belpoggi[3], Jia Chen[2] & Lauren Petrick[2,6*]

STAGE I: publications

Dr Michael Antoniou and his staff; joint publications with the Ramazzini Institute

Istituto Ramazzini
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Use of Shotgun Metagenomics and Metabolomics to Evaluate the Impact of Glyphosate or Roundup MON 52276 on the Gut Microbiota and Serum Metabolome of Sprague-Dawley Rats

Robin Mesnage,[1] Maxime Teixeira,[2] Daniele Mandrioli,[3] Laura Falcioni,[3] Quinten Raymond Ducarmon,[4] Romy Daniëlle Zwittink,[4] Francesca Mazzacuva,[5] Anna Caldwell,[5] John Halket,[5] Caroline Amiel,[2] Jean-Michel Panoff,[2] Fiorella Belpoggi,[3] and Michael Nicolas Antoniou[1]

[1]Gene Expression and Therapy Group, Department of Medical and Molecular Genetics, King's College London, Faculty of Life Sciences & Medicine, Guy's Hospital, London, UK
[2]Unité de Recherche Aliments Bioprocédés Toxicologie Environnements, University of Caen Normandy, Caen, France
[3]Ramazzini Institute, Bologna, Italy
[4]Center for Microbiome Analyses and Therapeutics, Leiden University Medical Center, Leiden, Netherlands
[5]Mass Spectrometry Facility, King's College London, London, UK

Environ Health Perspect. **129**: 17005, 2021
https://ehp.niehs.nih.gov/doi/10.1289/EHP6990?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%20%200pubmed

In-depth comparative toxicogenomics of glyphosate and Roundup herbicides: histopathology, transcriptome and epigenome signatures, and DNA damage

Robin Mesnage[1], Mariam Ibragim[1], Daniele Mandrioli[2], Laura Falcioni[2], Fiorella Belpoggi[2], Inger Brandsma[3] , Emma Bourne[4], Emanuel Savage[4], Charles A Mein[4], Michael N Antoniou[1*]

1 Gene Expression and Therapy Group, King's College London, Faculty of Life Sciences & Medicine, Department of Medical and Molecular Genetics, Guy's Hospital, London, SE1 9RT, UK.

2 Cesare Maltoni Cancer Research Center, Ramazzini Institute (RI), Via Saliceto, 3, 40010 Bentivoglio, Bologna, Italy.

3 Toxys, Robert Bolyeweg 4, 2333 CG, Leiden, The Netherlands

4 Genome Centre, Barts and the London School of Medicine and Dentistry, Blizard Institute, London E1 2AT, United Kingdom.

STAGE I: publications

Dr. Jia Chen and her staff

Environmental Pollution 280 (2021) 117002

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Maternal urinary levels of glyphosate during pregnancy and anogenital distance in newborns in a US multicenter pregnancy cohort☆

Corina Lesseur [a], Patrick Pirrotte [b], Khyatiben V. Pathak [b], Fabiana Manservisi [c, d], Daniele Mandrioli [c], Fiorella Belpoggi [c], Simona Panzacchi [c], Qian Li [a], Emily S. Barrett [e], Ruby H.N. Nguyen [f], Sheela Sathyanarayana [g], Shanna H. Swan [a], Jia Chen [a, *]

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[b] Collaborative Center for Translational Mass Spectrometry, Translational Genomics Research Institute, Phoenix, AZ, USA
[c] Cesare Maltoni Cancer Research Center (CMCRC), Ramazzini Institute (RI), Via Saliceto, 3, 40010, Bentivoglio, Bologna, Italy
[d] Department of Veterinary Medical Sciences, University of Bologna, Italy
[e] Department of Biostatistics & Epidemiology, Rutgers School of Public Health, Piscataway, NJ, USA
[f] Department of Epidemiology & Community Health, University of Minnesota, Minneapolis, MN, USA
[g] Department of Pediatrics, University of Washington and Seattle Children's Research Institute, Seattle, WA, USA

STAGE II

INTEGRATED EXPERIMENTAL STUDY ON SUB-CHRONIC TOXICITY, CARCINOGENICITY, REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

AIMS:

- To go deeper into studying the most important parameters emerged from the pilot study;
- assess multiple toxicological parameters in a single protocol
- test human-equivalent doses;
- explore windows of biological susceptibility, in particular for endocrine disruptive end-points

STAGE II: experimental plan

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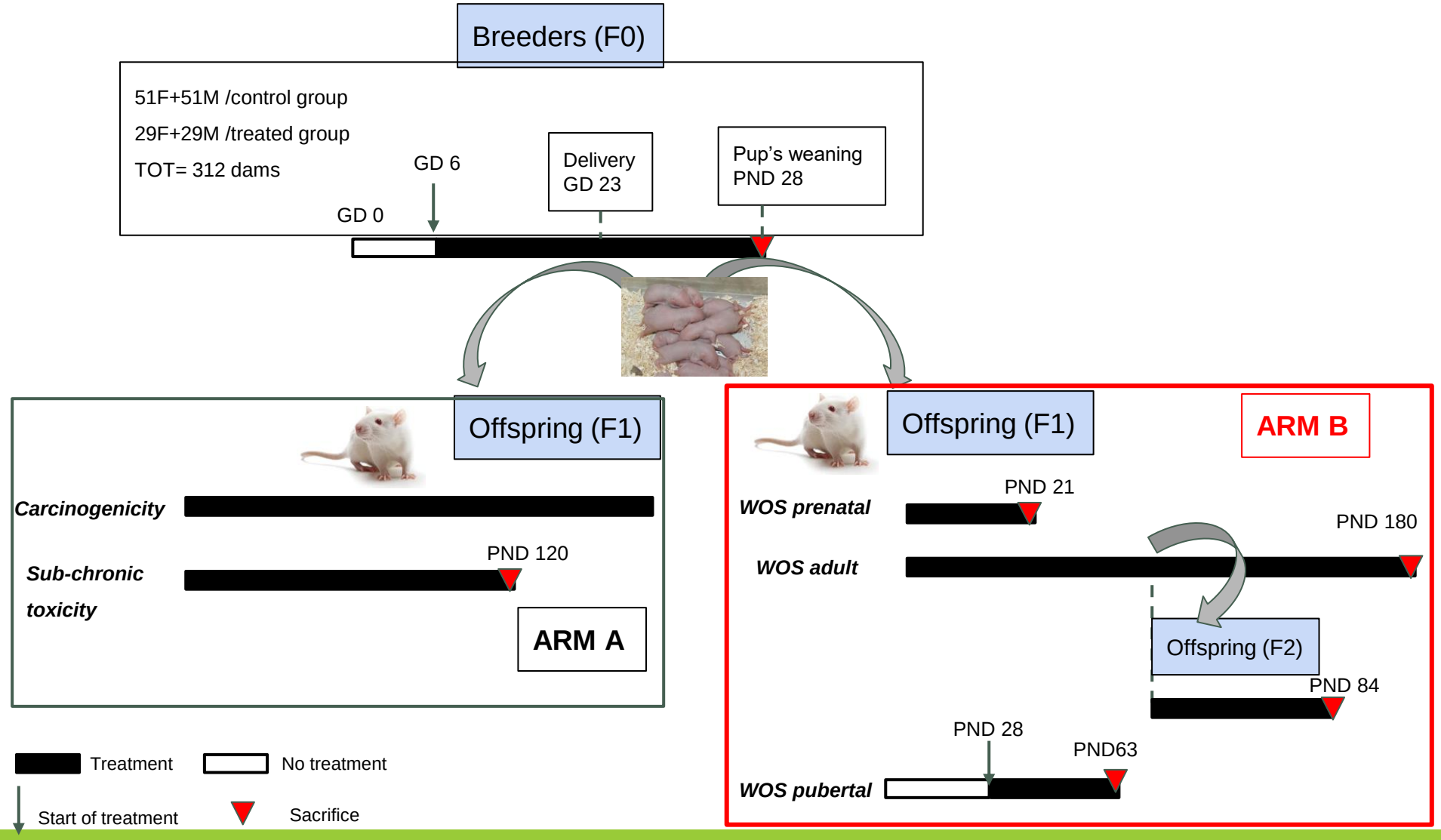
GROUP	TREATMENT	DOSES
I	Drinking water (DW)	-
II-IV	DW + Glyphosate	0 - 0.5 (ADI EU) – 5 – 50 (NOAEL EU) mg/Kg bw/day
V-VII	DW + Roundup Bioflow	0 -0.5 (ADI EU) – 5 – 50 (NOAEL EU) mg/Kg bw/day Glyphosate equivalent

- **Route of Administration:** *ad libitum* in drinking water
- **Test Substances:** Glyphosate (purity > 99.5%); Roundup Bioflow.

STAGE II: experimental design (OECD guidelines, GLP standard operating procedures



Global Glyphosate study: why we need to publish the data before the end of 2021?

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- No further robust data with GLP methods were produced since last approval in 2017.
- Glyphosate could be renewed as indicated by the Assessment Group on Glyphosate conclusions.
- We must publish our results before the end of 2021.
- Data from the Ramazzini Institute **ARM B** study on both Glyphosate and GBHs include:
  - multi-generational effects
  - neurotoxic effects
  - endocrine disrupting effects
  - prenatal developmental toxicity effects
  - Effects on the microbiome

Global Glyphosate study

What we need in term of costs (human and material resources) in order to publish the data on the endocrine disruptive effects and other important toxicological end points?

The whole amount for concluding and publishing the study is about

1 million of Euros

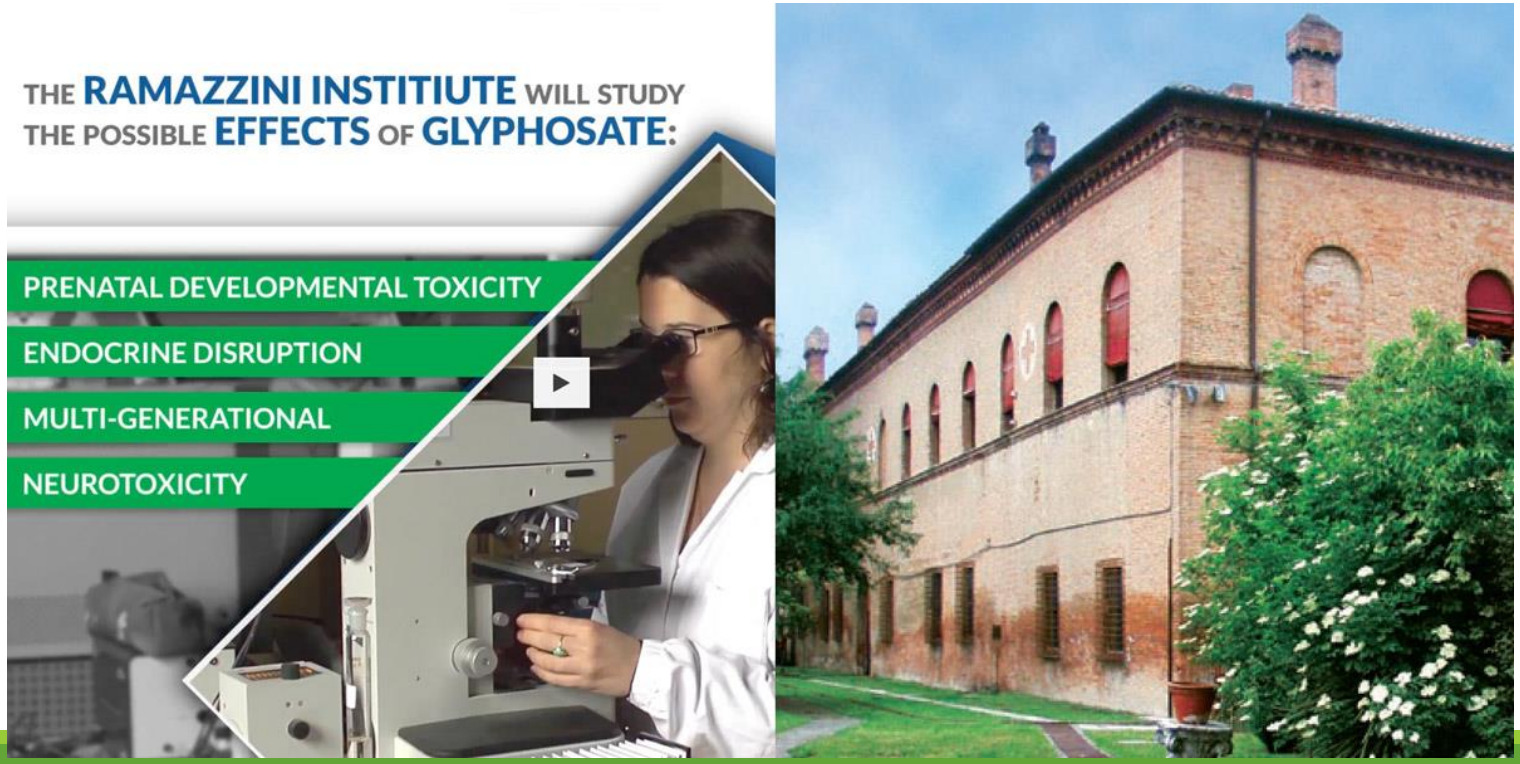
Global Glyphosate study: why we need to publish the data before the end of 2021?

The Global Glyphosate Study will supply valuable data of unprecedented power to enable **regulators, governments and the general public** of every Country to answer the questions:

- could glyphosate be re-authorized in 2022?
- Is it really safe when we consider end-points other than cancer?

Global Glyphosate study: conclusions

Whatever the outcome of the Ramazzini Institute study, the findings will provide regulatory agencies and policy-makers with **solid independent results obtained by a shared research project** on which they can confidently base their risk assessments and their evaluations, including the upcoming **decision for the reauthorization for glyphosate use in Europe in 2022**.



Thank you!