



The European Partnership
for Alternative Approaches to Animal Testing

EPAA 3Rs Awards and Grants

2021

REFINEMENT PRIZE

2021

HaPILLnes

S

PRECISE VOLUNTARY ORAL DRUG DOSING IN RODENTS - AN INNOVATIVE 3Rs
APPROACH



ICBR Coimbra Institute for Clinical
and Biomedical Research

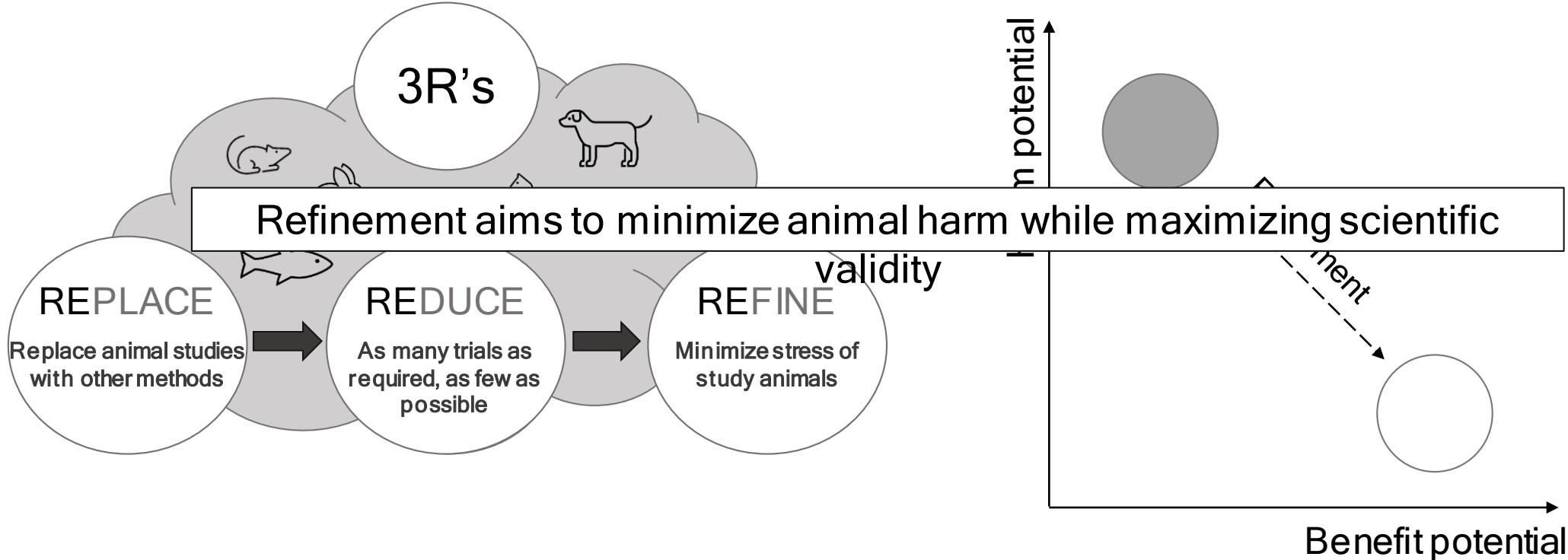


FACULDADE DE MEDICINA
UNIVERSIDADE D
COIMBRA

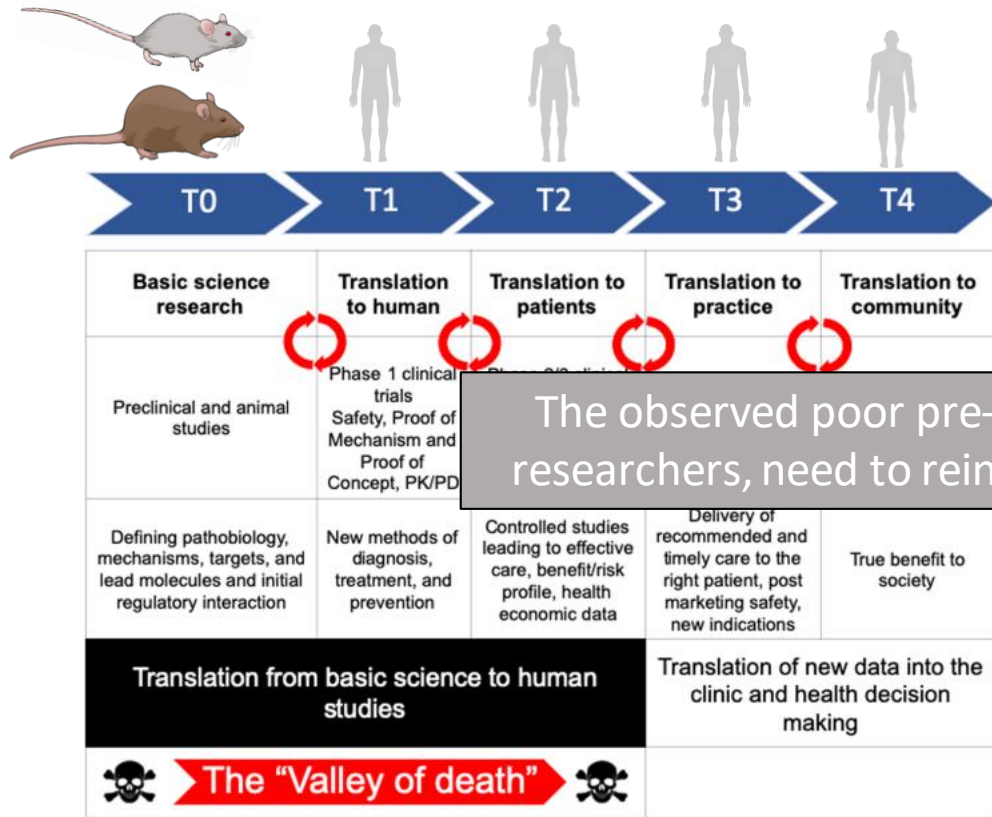


Laboratory Animal Sciences

The science and technology dealing with the procurement, breeding, care, health, and selection of animals used in biomedical research and testing.

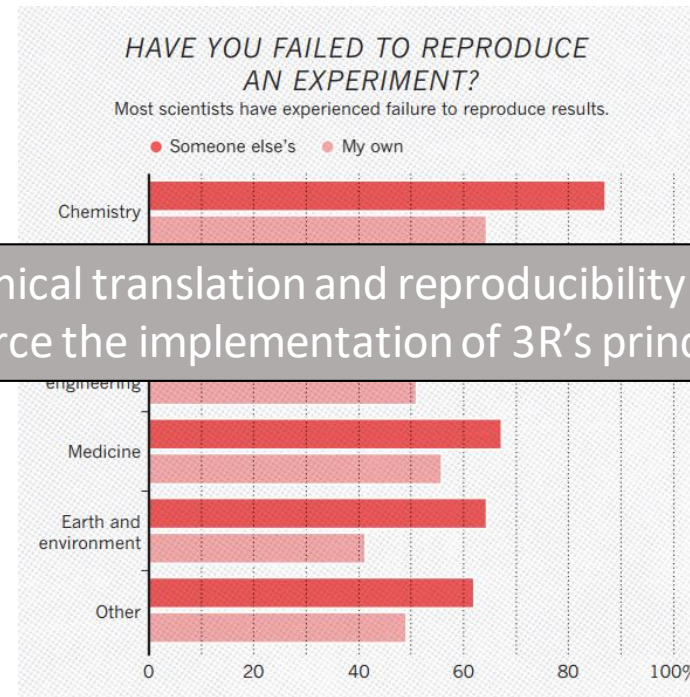


TRANSLATION & REPRODUCIBILITY CRISIS

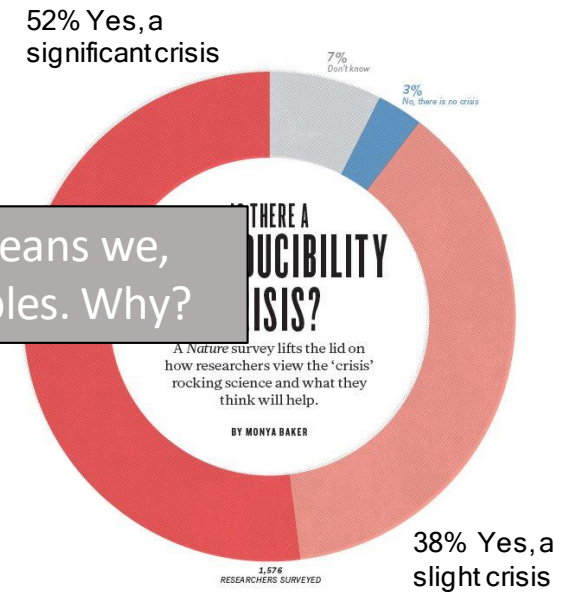


Adapted from: Seyhan et al 2019

1576 researchers surveyed



The observed poor pre-clinical translation and reproducibility means we, researchers, need to reinforce the implementation of 3R's principles. Why?



Adapted from: <https://www.nature.com/articles/533452a>



Concept Paper

The Role of the Three Rs in Improving the Planning and Reproducibility of Animal Experiments

Adrian J. Smith ^{1,*} and Elliot Lilley ²



European Journal of Pharmacology

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

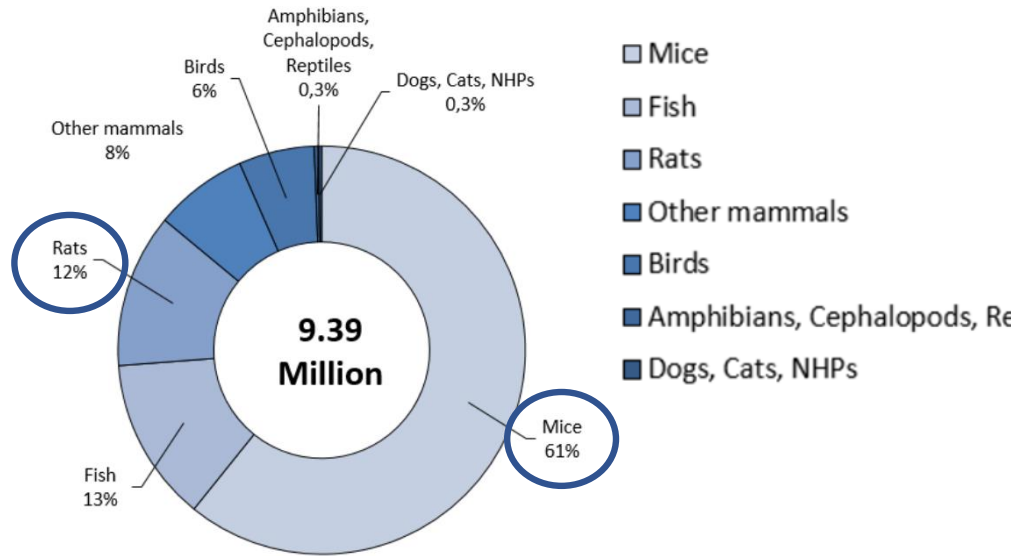


Review

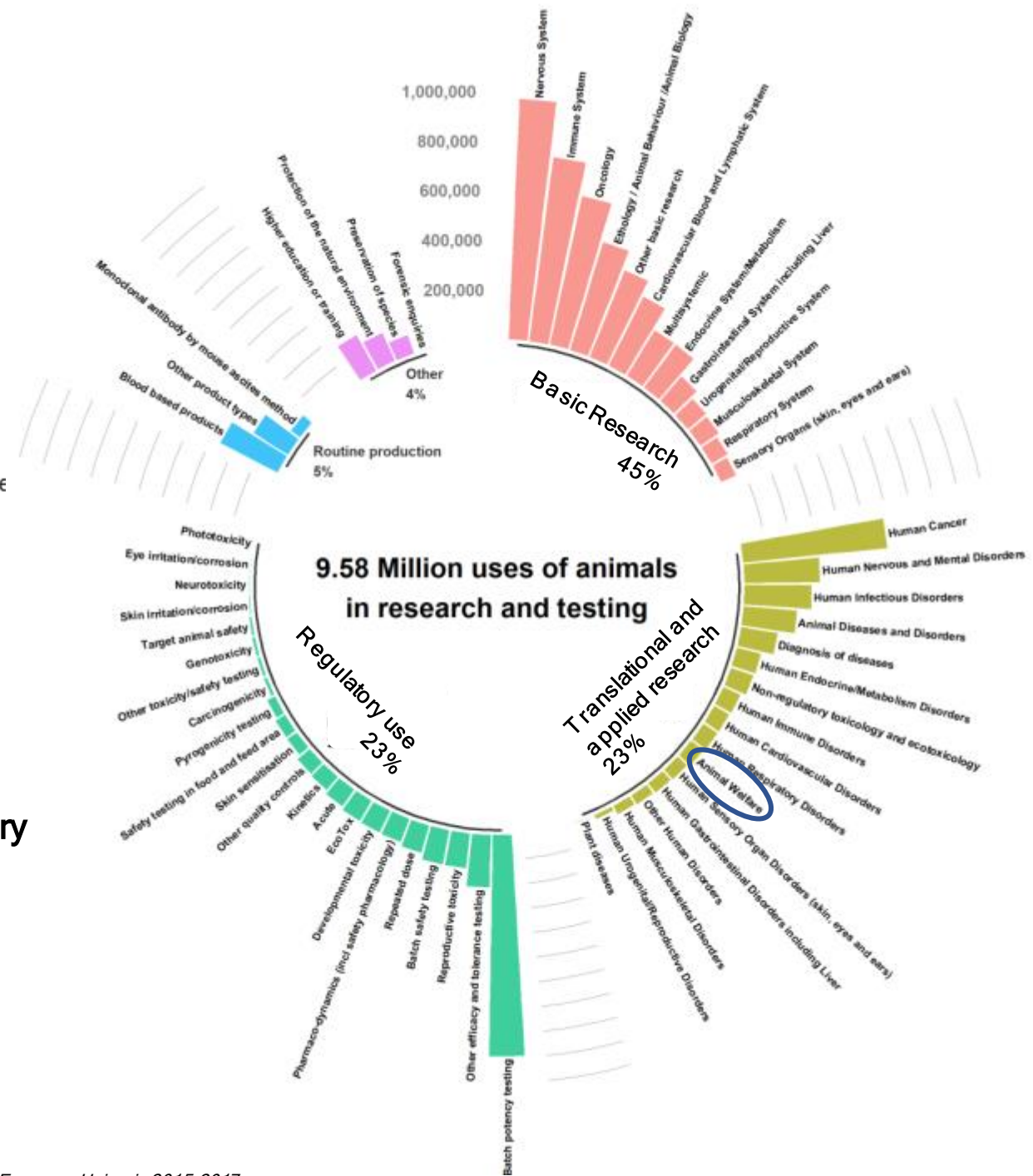
The multifactorial role of the 3Rs in shifting the harm-benefit analysis in animal models of disease

Melanie L. Graham ^{a,b,*}, Mark J. Prescott ^c





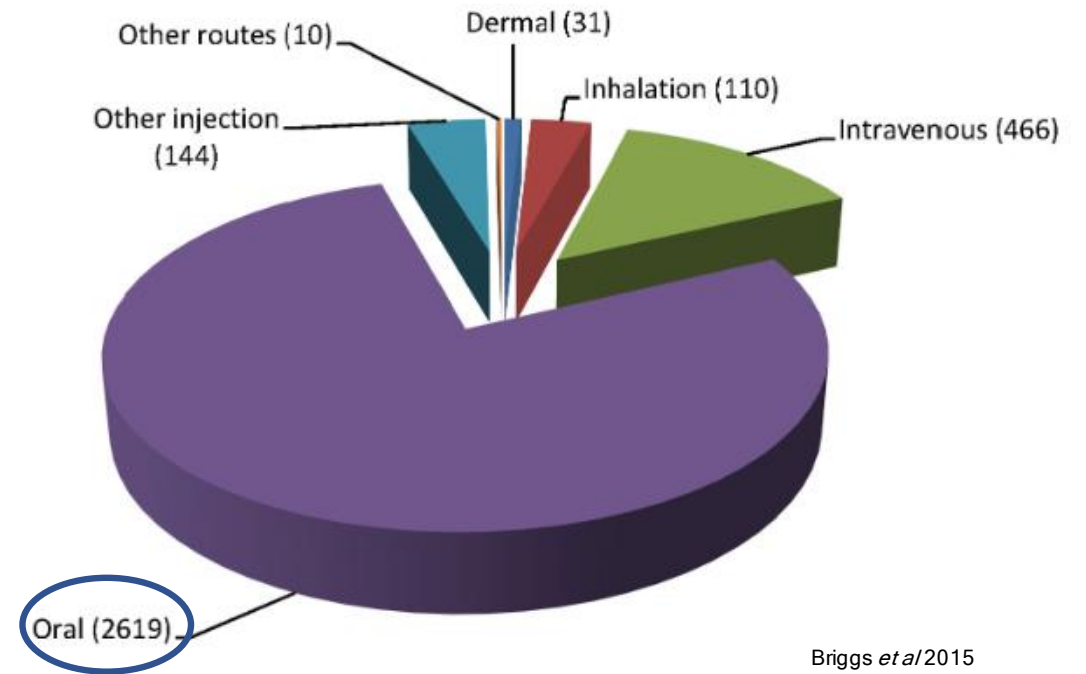
Because \approx 9 million animals are used in E.U. every year for research purposes



In humans



In pre-clinical studies



Briggs *et al*/2015

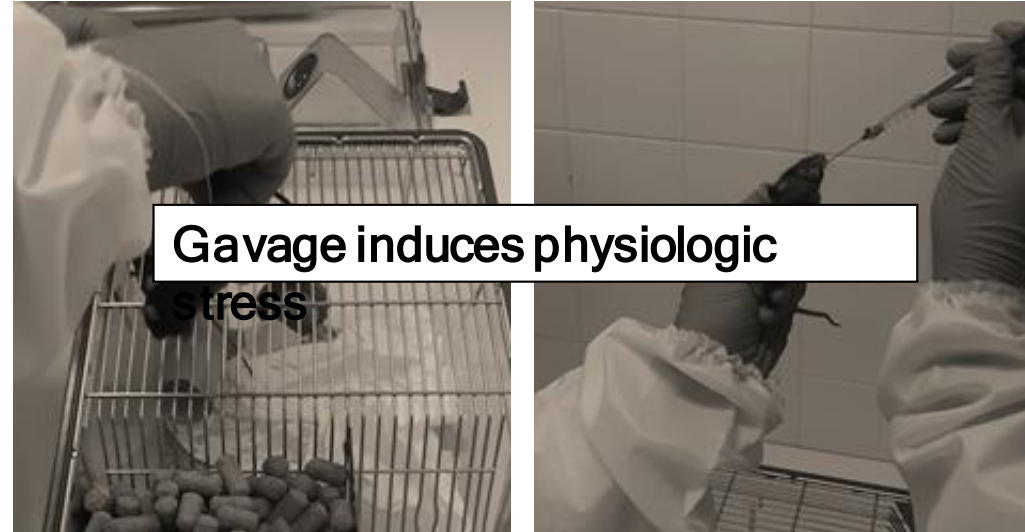
Oral dosing is part of the daily routine for millions of laboratory animals

Nowadays:

GAVAGE



1. **Restrain** the animal
2. **Insert** cannula into the animal's stomach
3. **Press** the syringe plunger to **inject** the drug to the animal's stomach



Gavage induces physiologic stress

Stress Produced by Gavage Administration in the Rat

ALAN P. BROWN, PHD, DABT, NANCY DINGER, BS, AND BARRY S. LEVINE, DSC, DABT

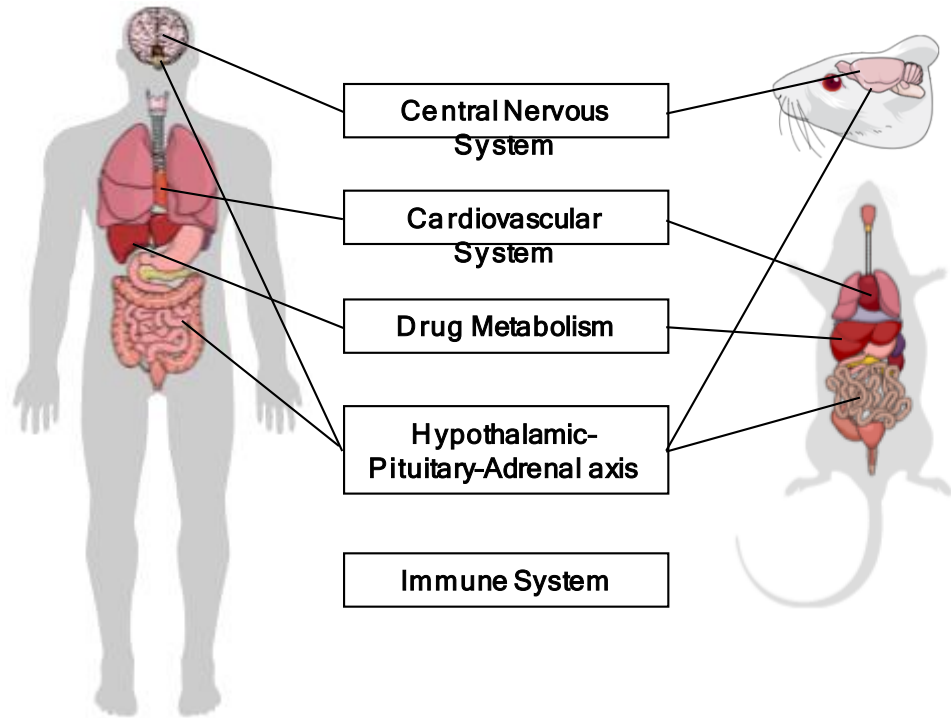
Does the Stress of Laboratory Life and Experimentation on Animals Adversely Affect Research Data? A Critical Review

Jarrold Bailey

Gavage-Related Reflux in Rats: Identification, Pathogenesis, and Toxicological Implications (Review)

SIEGRID DAMSCH¹, GARY EICHENBAUM², ALFRED TONELLI², LIEVE LAMMENS¹, KATHLEEN VAN DEN BULCK¹, BIANCA FEYEN¹,
JOHN VANDENBERGHE¹, ANTON MEGENS¹, ELAINE KNIGHT², AND MICHAEL KELLEY²

Gavage induces physiologic stress



Thus, gavage conditions the observed metrics in pre-clinical studies

Regulation of adult neurogenesis in the hippocampus by stress, acetylcholine and dopamine

J. Veena,
B. S. Shankaranarayana
Rao¹, B. N. Srikumar²

Scientific and Regulatory Policy Committee Review

Interpreting Stress Responses during Routine Toxicity Studies: A Review of the Biology, Impact, and Assessment

NANCY E. EVERDS¹, PAUL W. SNYDER², KEITH L. BAILEY³, BRAD BOLON⁴, DIANNE M. CREASY⁵, GEORGE L. FOLEY⁶,
THOMAS J. ROSOL⁷, AND TERESA SELLERS⁸

OPEN

The role of the microbiome and psychosocial stress in the expression and activity of drug metabolizing enzymes in mice

Nina Zemanová¹, Pavel Anzenbacher², Iveta Zapletalová², Lenka Jourová^{1,3,4},
Petra Hermanová³, Tomáš Hudcovic³, Hana Kozáková³, Martin Vodička⁴, Jiří Pácha⁴ &
Eva Anzenbacherová¹

SCIENTIFIC
REPORTS
nature research

Neurobiology of Disease

Stress-Induced Recruitment of Bone Marrow-Derived Monocytes to the Brain Promotes Anxiety-Like Behavior

Eric S. Wohleb,^{1,2} Nicole D. Powell,¹ Jonathan P. Godbout,^{2,3,4} and John F. Sheridan^{1,3,4}

Clinical and Developmental Immunology

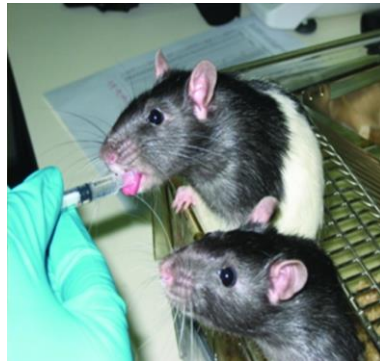
Review Article

Neuroendocrine Immunoregulation in Multiple Sclerosis

Nathalie Deckx, Wai-Ping Lee, Zwi N. Berneman, and Nathalie Cools

Over the years, many alternatives have been developed such as:

Drug suspension in sucrose solution and voluntary syringe feeding



[Atcha et al. 2010](#)

Fast dissolving oral drug biofilm and voluntary syringe feeding



[Yardley et al. 2015](#)

Drug given in highly caloric and palatable substances (e.g. Nutella, peanut butter, sugar paste)



Drug given in food/drinking water



[jko et al. 2017](#)

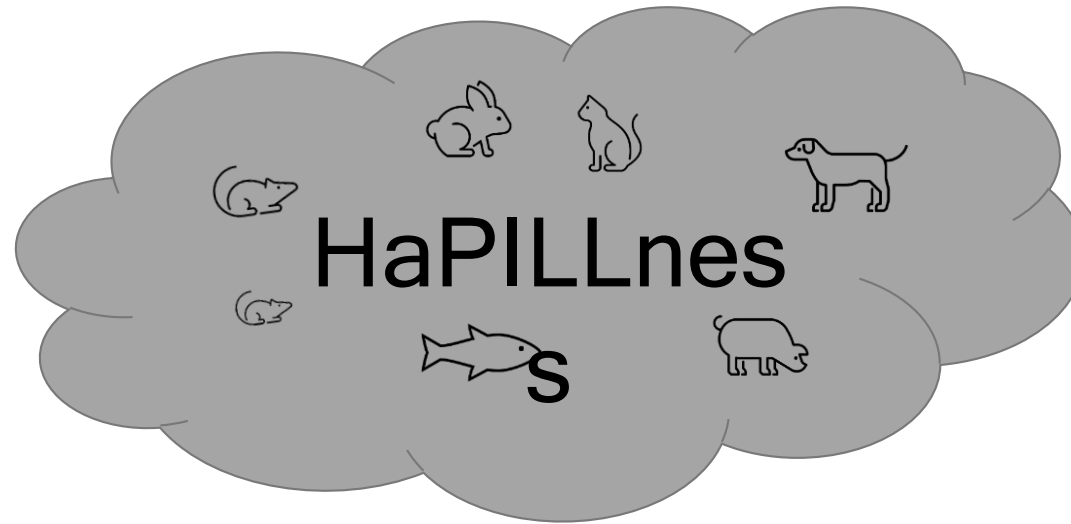
Limitations

- Lack of dose precision
- Requires single-housing
- Requires animal restraint
- Metabolically disruptive



[Sobolewski et al. 2016](#)

So, we proposed ourselves to find a truly voluntary, stress free and metabolic inert alternative



To help implement the 3R's principles of Refinement and Reduction in oral drug dosing



1

PILL TECH VOLUNTARY CONSUMPTION

2

PRECISE ORAL DOSING

3

STRESS FREE

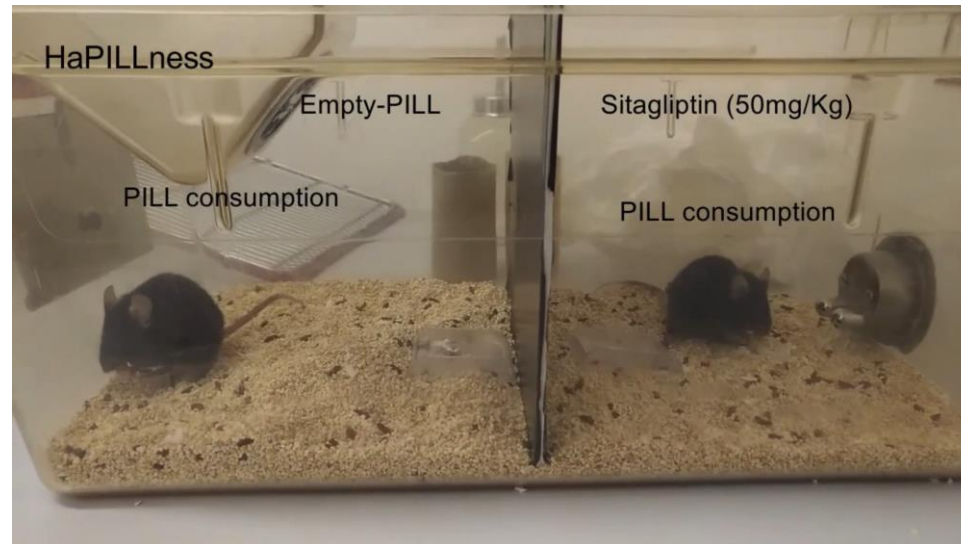
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METABOLIC INOCUITY

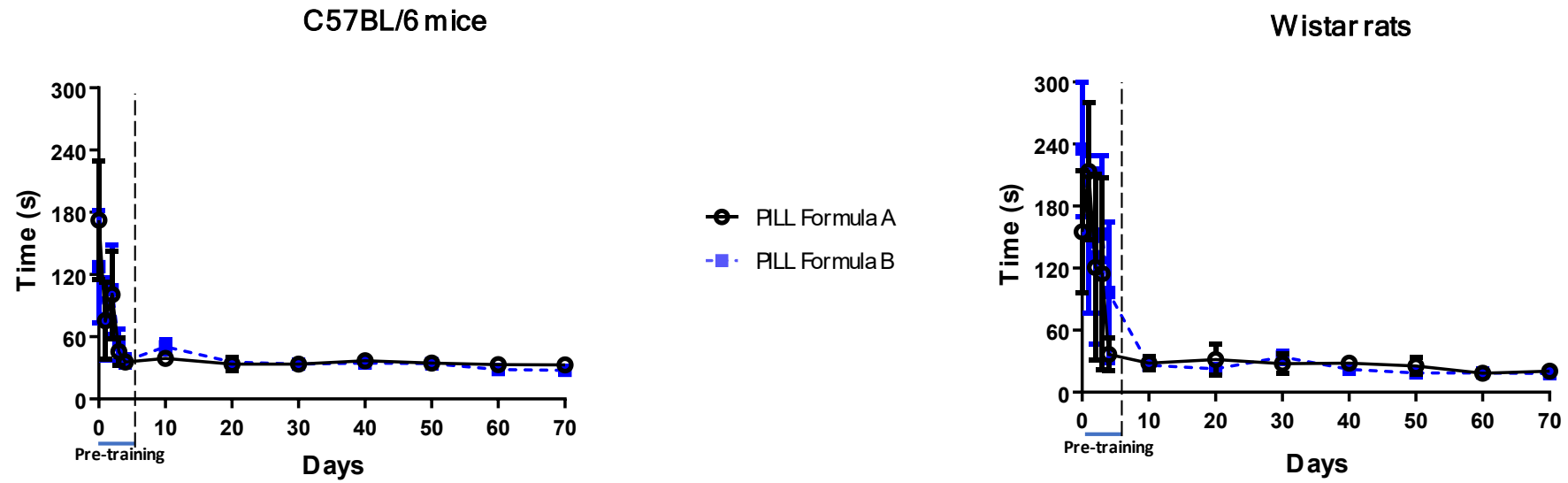
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SAFETY

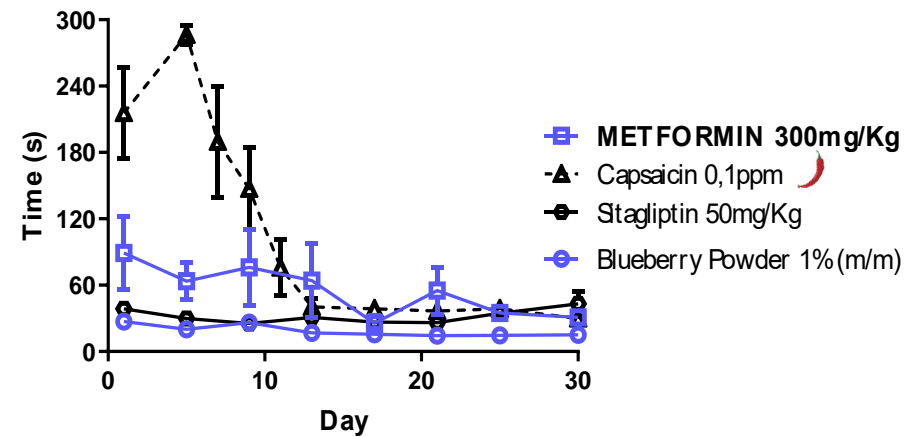
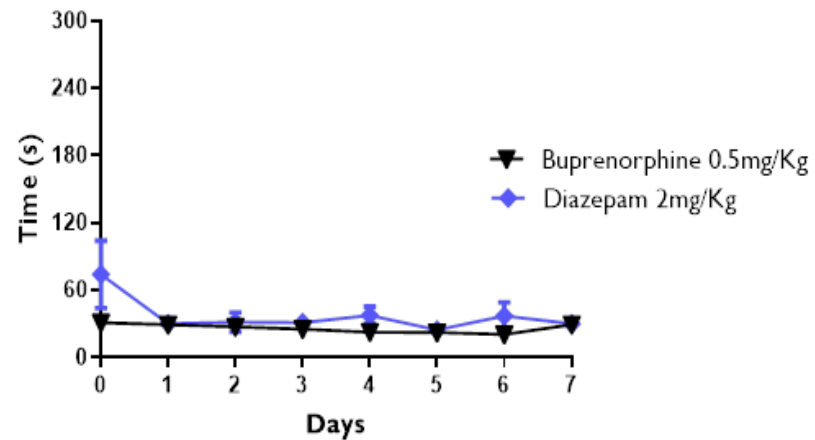
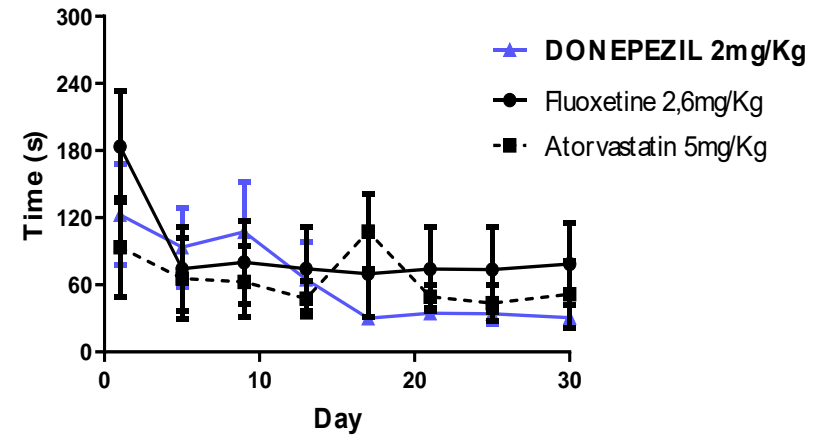
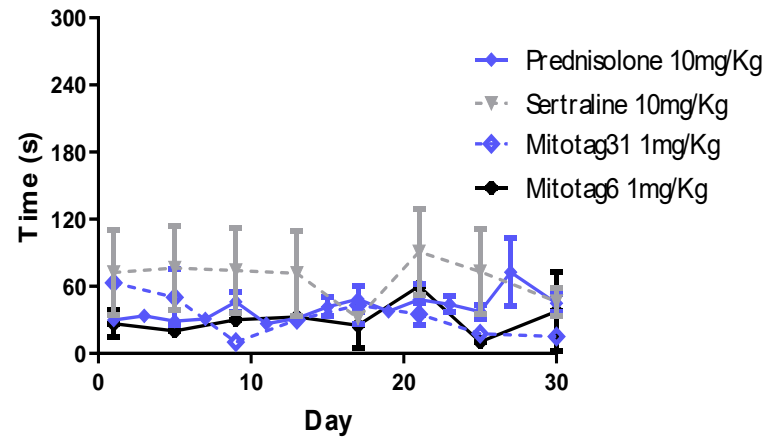
PILL TECH VOLUNTARY CONSUMPTION



PILL TECH VOLUNTARY CONSUMPTION



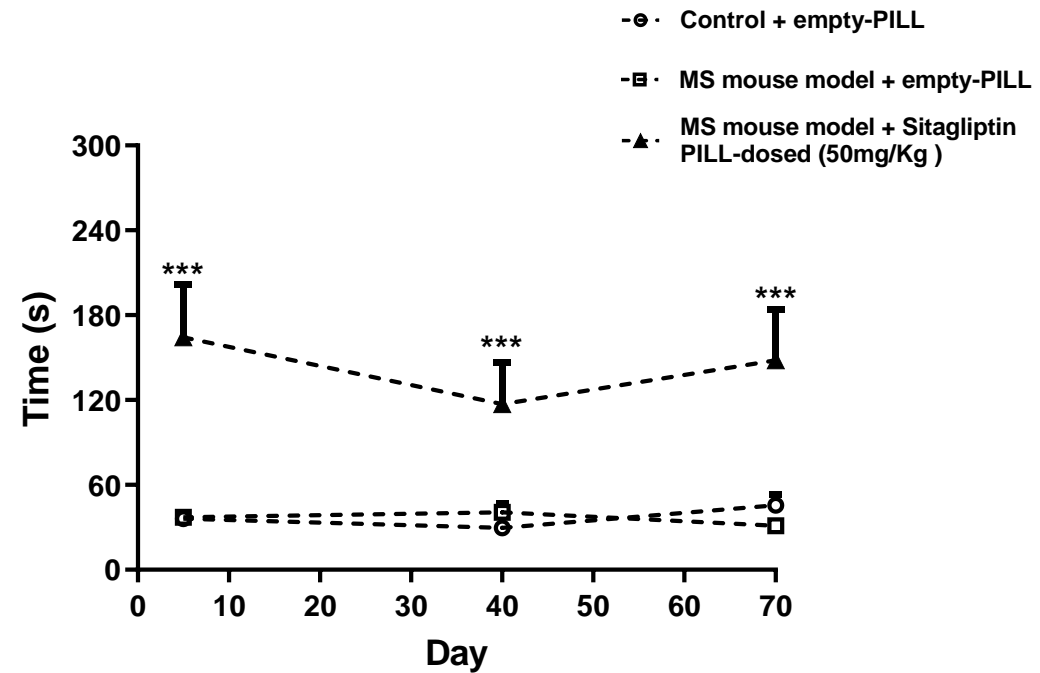
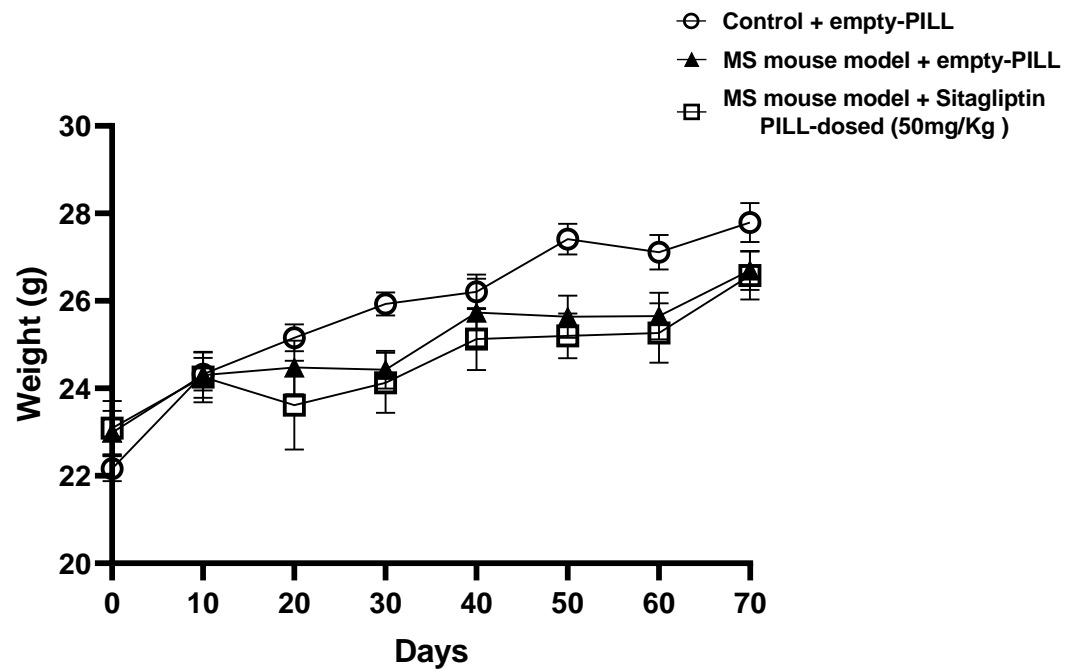
PILL TECH VOLUNTARY CONSUMPTION



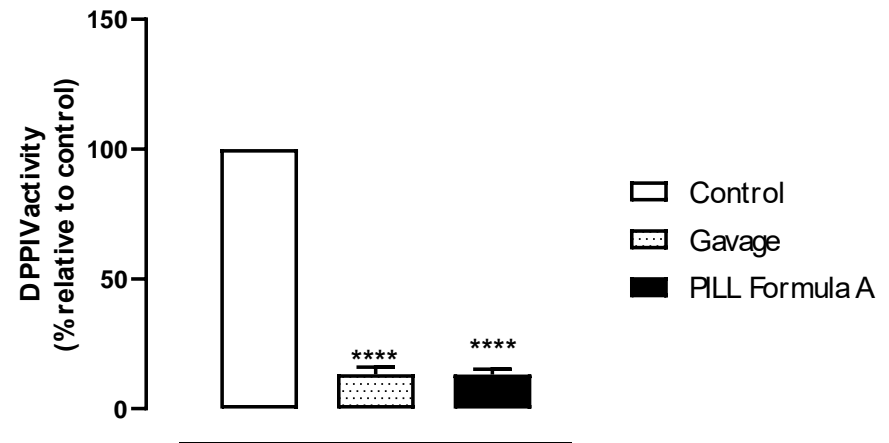
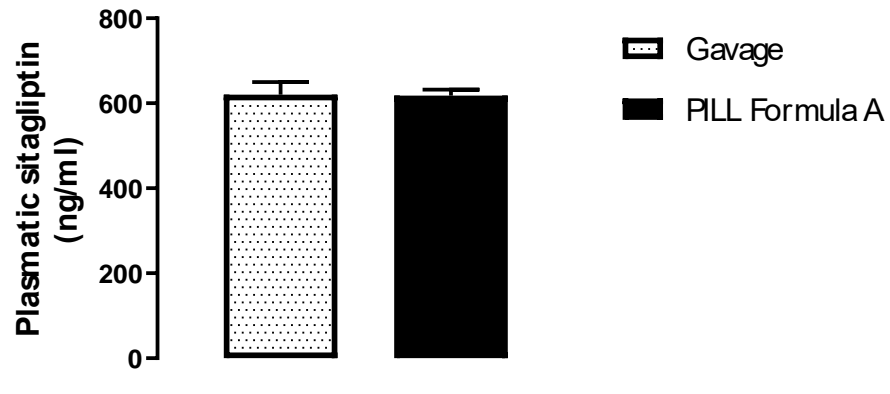
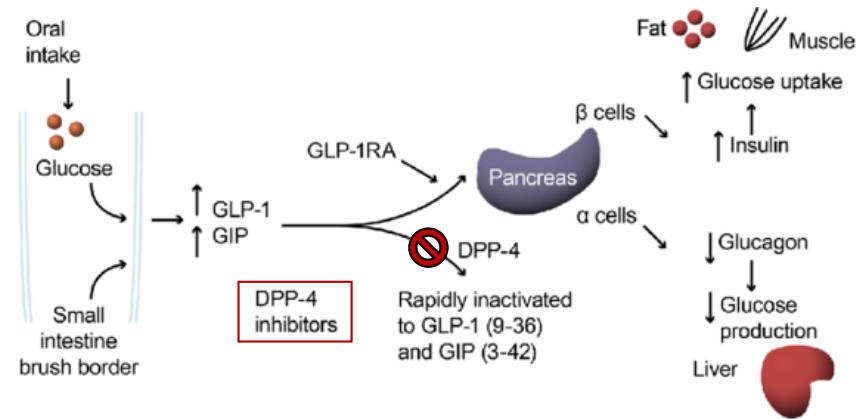
PILL TECH VOLUNTARY CONSUMPTION

Animal model of disease:

cuprizone intoxication multiple sclerosis model



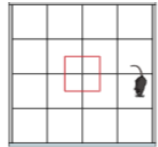
PRECISE ORAL DOSING



C57BL/6 mice (n= 5 experimental group). ANOVA oneway. **** p>0.0001 vs control.

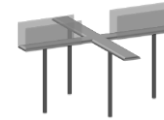
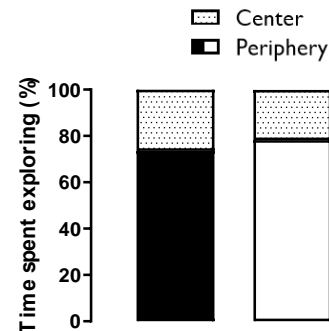
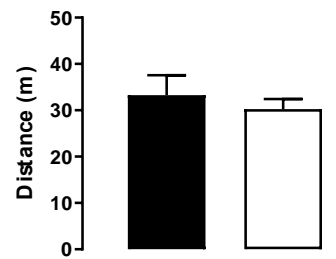
Data are presented as mean ± s.e.m.

STRESS FREE



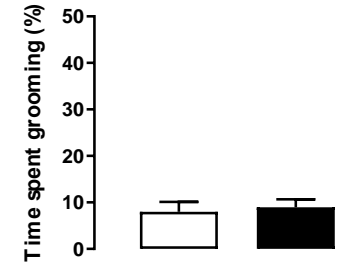
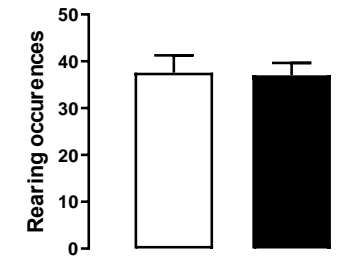
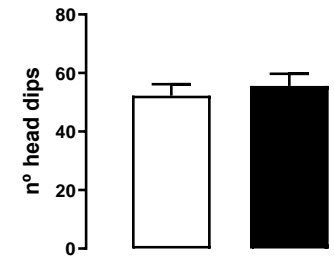
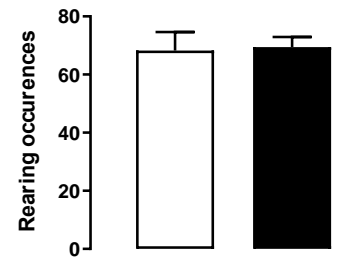
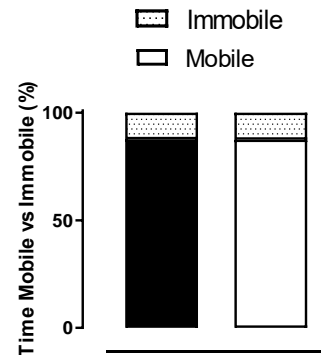
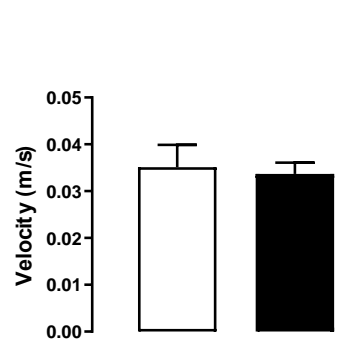
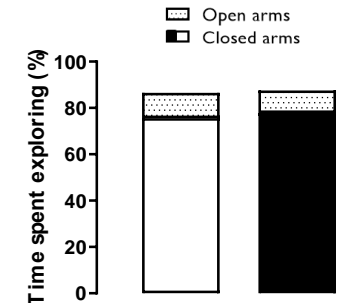
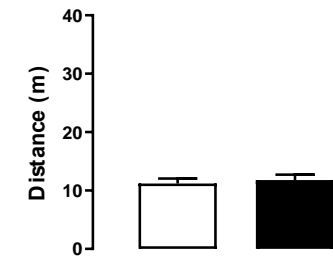
Open Field Test

■ Control
□ PILL Formula A

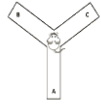


Elevated Plus Maze

■ Control
□ PILL Formula A



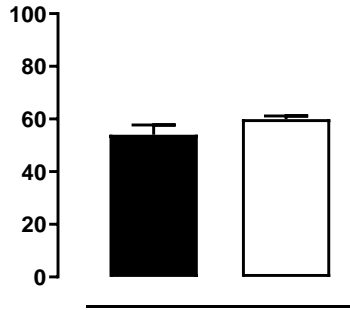
STRESS FREE



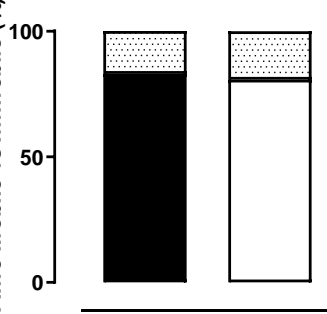
Y-Maze test

■ Control
□ PILL Formula A

Percentage of spontaneous alternation (%)



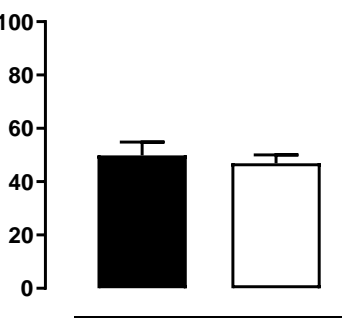
Time Mobile vs Immobile (%)



Splash test

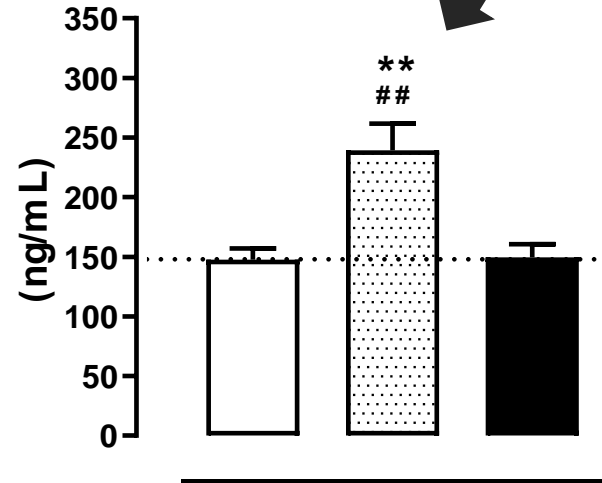
■ Control
□ PILL Formula A

Time spent grooming (%)



Direct physiologic readout

Plasmatic Corticosterone (ng/mL)



□ Control
▤ Gavage
■ PILL Formula A

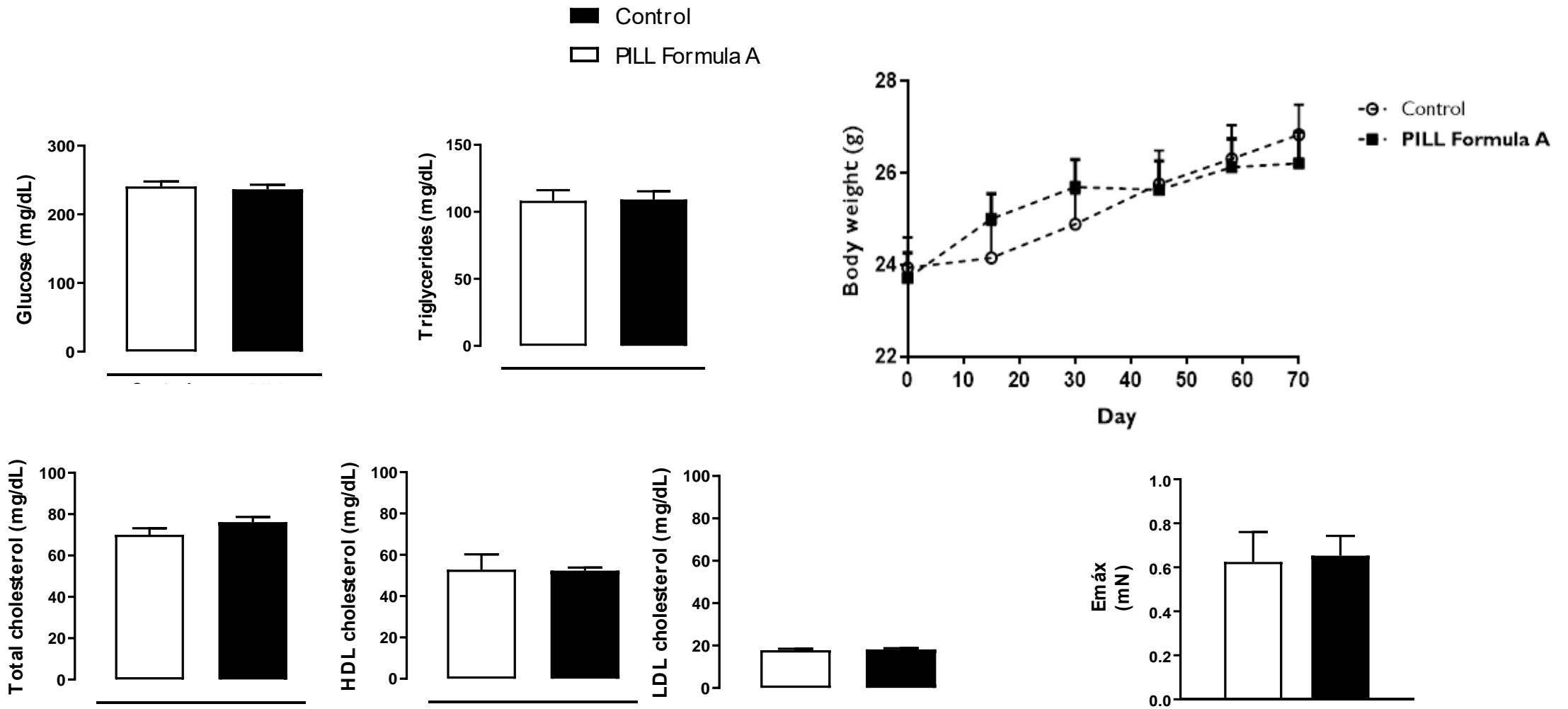
Acute: 30min postgavage

C57BL/6 mice (n= 5 experimental group). ANOVA oneway. **p>0.01 vs control, ## p>0.01 vs PILL.

C57BL/6 mice (n= 6 experimental group). Data are presented as mean ± s.e.m.

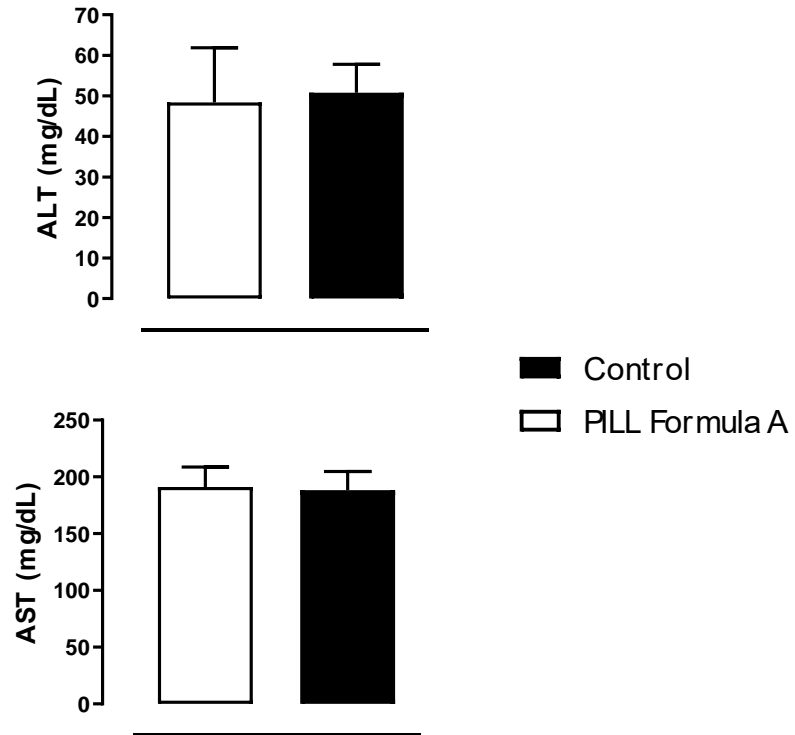
PILL.

METABOLIC INOCUITY

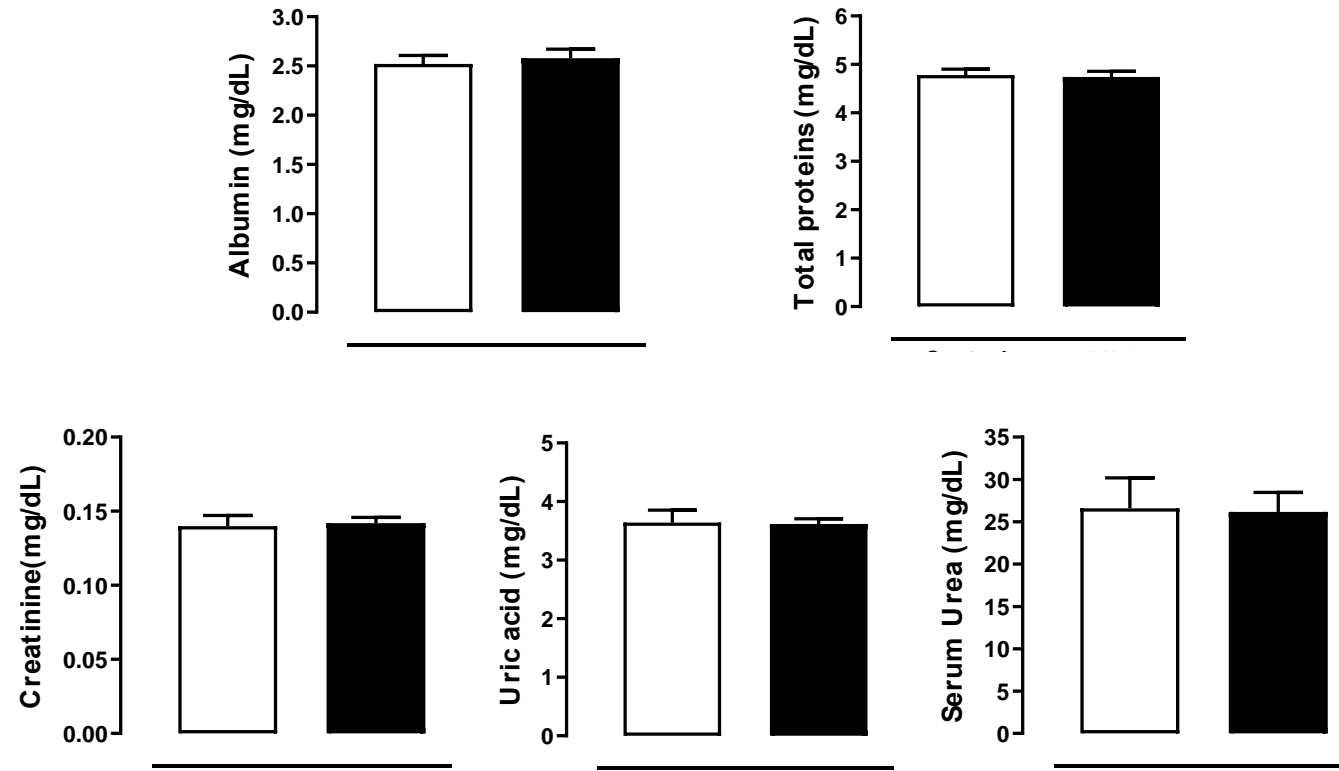


SAFETY

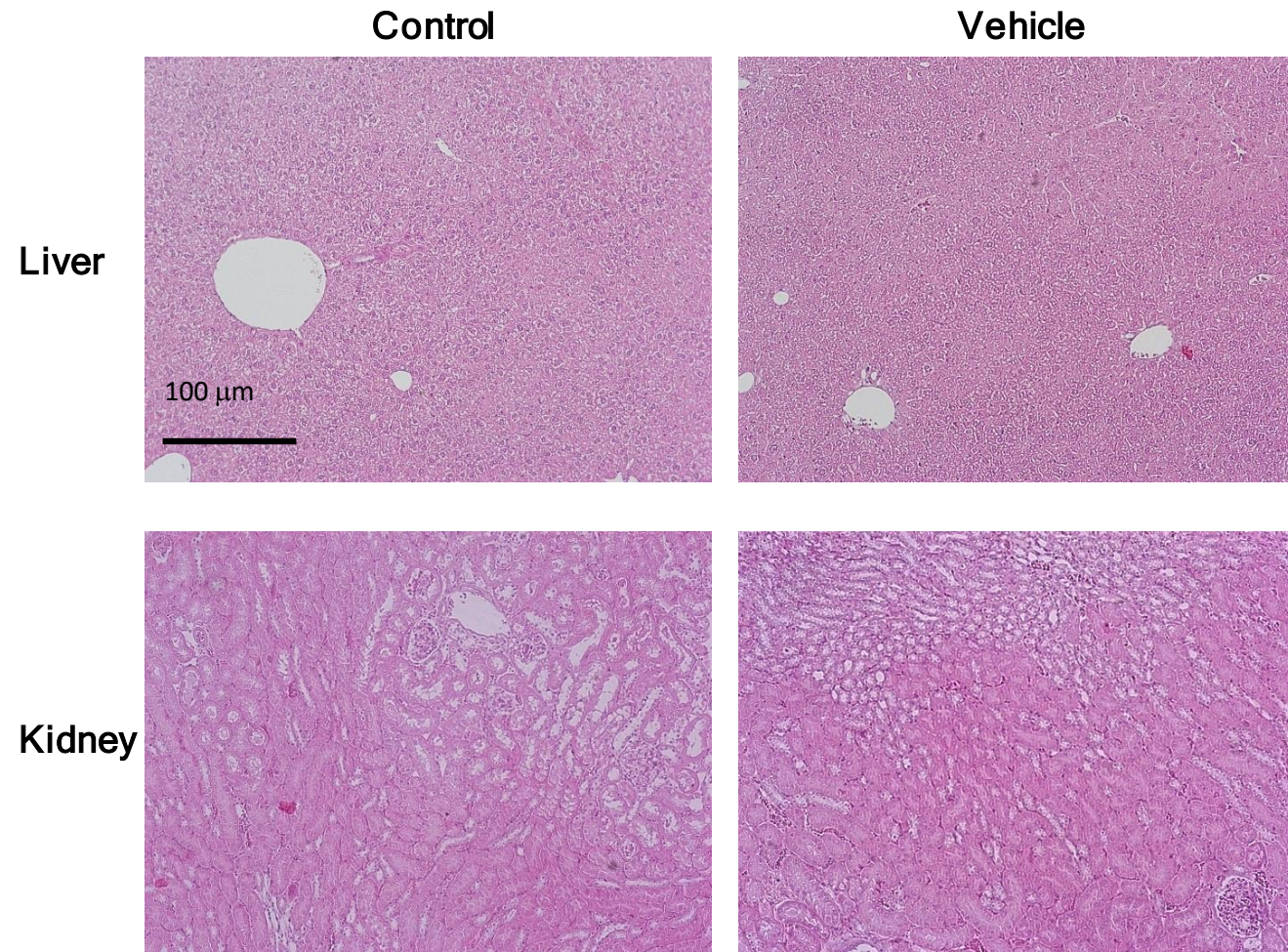
Liver function profile



Kidney function profile



Histology of liver and kidney sections



PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

To: **PCT**

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (PCT Rule 43bis.1)

see form PCT/ISA/220

Date of mailing (day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No. PCT/IB2021/053124	International filing date (day/month/year) 15.04.2021	Priority date (day/month/year) 27.04.2020
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International Patent Classification (IPC) or both national classification and IPC
INV. A61K9/20 A23K10/00 A61K31/40 A61K31/4985 A61K31/505 A01N25/08 A01P11/00

Applicant
UNIVERSIDADE DE COIMBRA

Prior art documents

1 Reference is made to the following documents:

- D1 EP 0 772 391 A1 (ZENECA LTD [GB]) 14 May 1997 (1997-05-14)
- D2 US 2005/181003 A1 (ENDEPOLIS STEFAN [DE] ET AL) 18 August 2005 (2005-08-18)
- D3 DHAWAN SANDEEP S ET AL: "Oral dosing of rodents using a palatable tablet", PSYCHOPHARMACOLOGY, vol. 235, no. 5, 6 March 2018 (2018-03-06), pages 1527-1532, cited in the application
- D4 RAYA JULIANA ET AL: "Corticosterone Assimilation by a Voluntary Oral Administration in Palatable Food to Rats", JOURNAL OF APPLIED ANIMAL WELFARE SCIENCE, vol. 22, no. 1, 11 May 2018 (2018-05-11), pages 37-41

Re Item V

Novelty (Article 33(1) In the sense of Article 33(2) PCT)

Consequently, the subject-matter of Claims 1-15 can be considered novel in the sense of Article 33(2) PCT.

Inventive Step (Article 33(1) in the sense of Article 33(3) PCT)

Since there was no hint or suggestion towards the specific formulation of Claim 1 and since the demonstrated high voluntary acceptance of the formulation was not obviously derivable from any of the prior art documents or their combinations, it appears that an inventive step can be acknowledged for the subject-matter of Claim 1.

For the same reason, the subject-matter of Claims 2-15 also appears to involve an inventive step.

Consequently, the subject-matter of Claims 1-15 appears to meet the requirements of Article 33(3) PCT.



Sofia Viana, PhD
Inventor/supervisor



Inês Preguiça,
HaPILLness PhD
student



Flávio Reis, PhD
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Sara Nunes, PhD student
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Funding: 2020.08560.BD

