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THE STUDY OF MODES OF ACTION: THE AOP

Ellen Fritsche

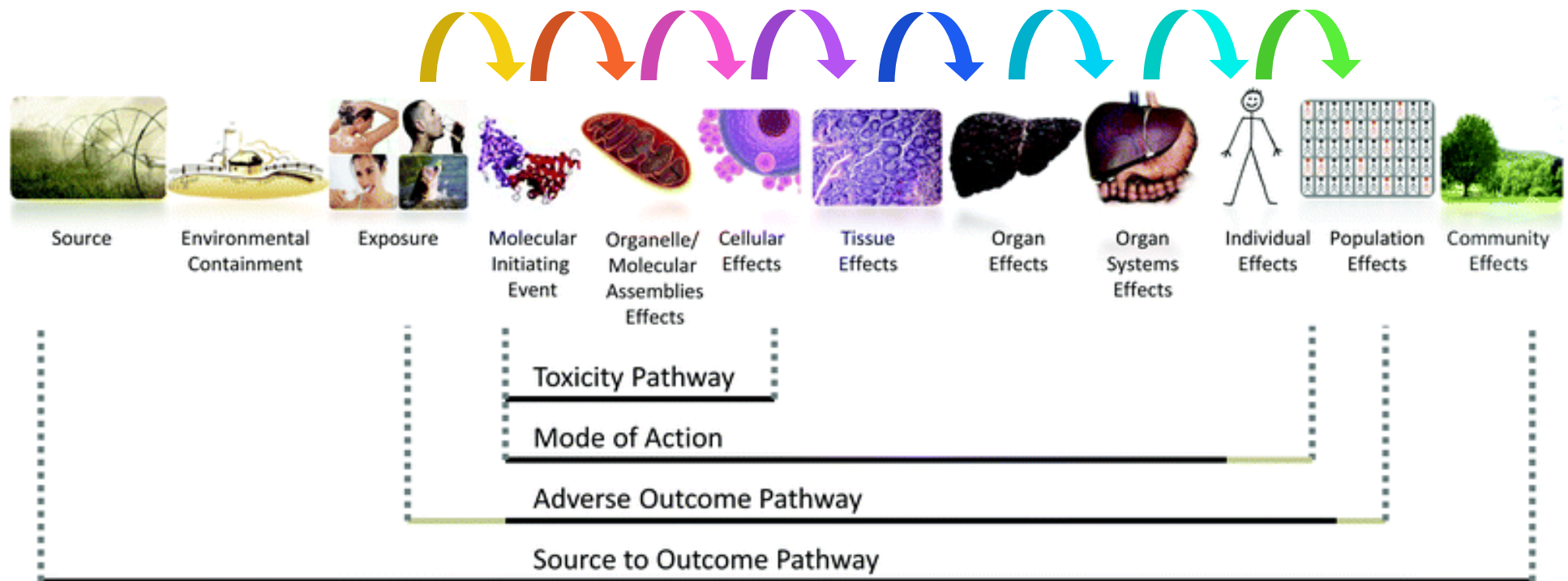
EFSA Working Group 6./7. October 2015

Mitglied der

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The Adverse Outcome Pathway (AOP) concept

Scientific Framework for the management of toxicological knowledge, information and data



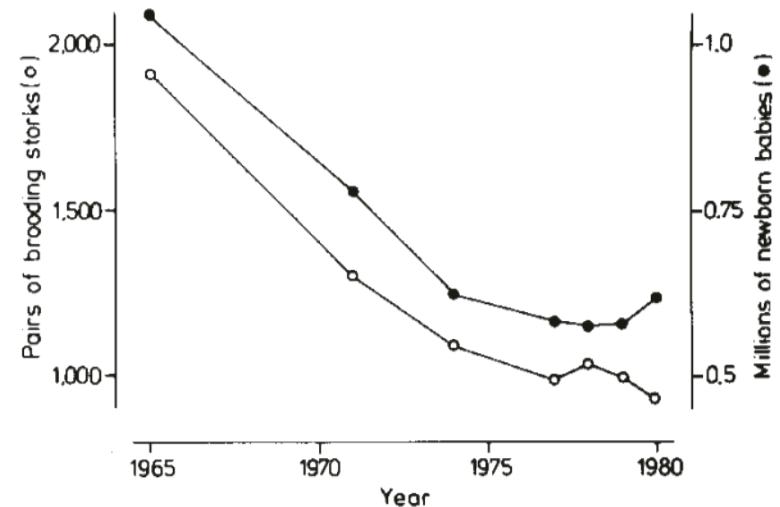
The Theory of Storks assessed with the AOP concept



500 babies/year/stork pair

A new parameter for sex education

SIR—There is concern in West Germany over the falling birth rate. The accompanying graph^{1,2} might suggest a solution that every child knows makes sense.



HELMUT SIES

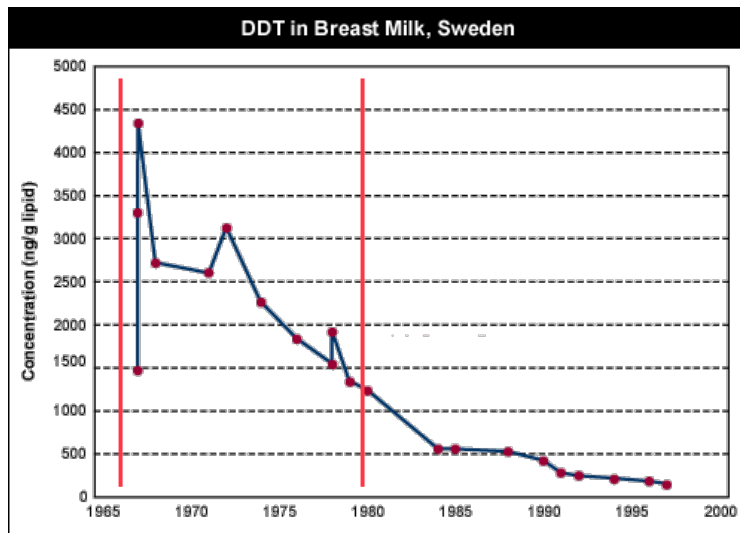
*Institut für Physiologische Chemie 1,
Universität Düsseldorf,
Moorenstrasse 5, D-4000 Düsseldorf,
FRG*

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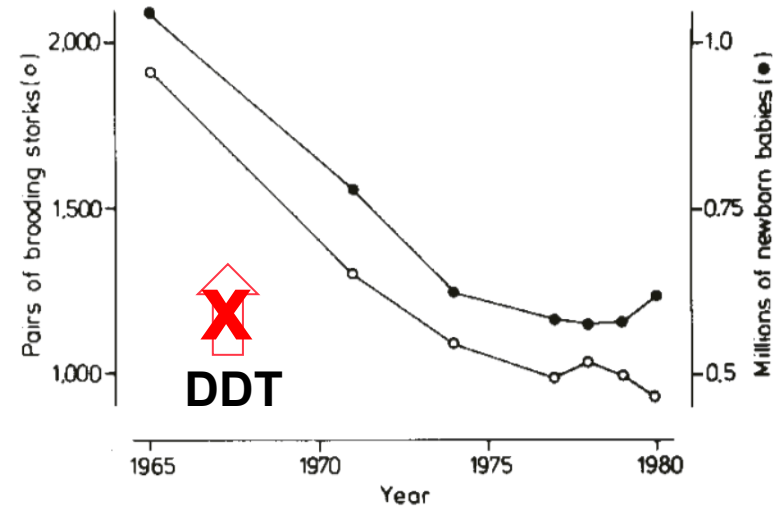
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Sies H, Nature 1988

The Theory of Storks assessed with the AOP concept



Source: NRDC



Leibniz

Sies H, Nature 1988

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The Theory of Storks assessed with the AOP concept



Hypothesis:

DDT exposure ↓



Stork population ↓



Babies ↓

The Theory of Storks assessed with the AOP concept



search: DDT AND stork

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Bull Environ Contam Toxicol. 2010 Aug;85(2):130-35. doi: 10.1007/s00128-010-0245-8. Epub 2010 Jun 23.

Lev

Dha

A

Abs

Con

the

from 11.4 ng/mL in White ibis *Threskiornis melanocephalus* to 286 ng/mL in Sarus Crane *Grus antigone*, while summation operatorDDT ranged between 19 ng/mL in Black Ibis *Pseudibis papillosa* and 147 ng/mL in Painted Stork *Mycteria leucocephala*. p,p'-DDE was accounted for more than 50% of total DDT in many of the samples analysed. However, a p,p'-DDT to p,p'-DDE ratio higher than one obtained for many species of birds indicates the recent use of DDT in this study. The highest DDT concentrations in the following order: granivores < insectivores. The DDT concentrations in the plasma of birds are not indicative of toxicity of organochlorine compounds. However, con

...summation operatorDDT 147 ng/mL in Painted Stork *Mycteria leucocephala*.

- Endocrine Disruptor
- Egg shell thinning
- Bird population decline

Similar articles

Distribution of persistent organochlorine chemical residues [Environ Monit Assess. 2011]

Accumulation pattern of persistent organochlorine [Environ Sci Pollut Res Int. 2013]

Organochlorine pesticide residues in eggs and tissues of hou [Bull Environ Contam Toxicol. 2...]

Lack of CAUSALITY defeats Theory of Storks



No literature support X

**Storks have rarely been
seen at sites of baby
delivery**

Hypothesis:

DDT exposure ↓

Stork population ↓

X

Babies ↓

Developmental effects of high dose plant extracts?

Food supplements contain high amounts of plant extracts or their pure ingredients.



General toxicities **lethal doses 50** are commonly known.

Consumers consider herbal-based food supplements as healthy and safe...

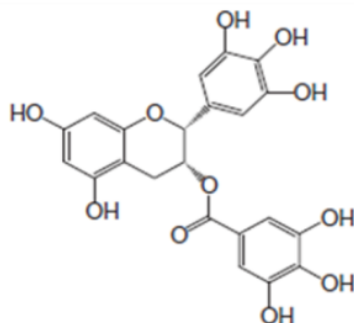


... and use them during pregnancy.

Moussally et al., 2009
Bishop et al., 2011
Forster et al., 2006
Nordeng et al., 2004

Developmental effects of high dose EGCG?

Epigallocatechin gallate **EGCG**



Potential health benefits in preventing or treating several chronic diseases, including cancer.

MoA:

**Cell proliferation
Cell adhesion
Cell migration**

Singh et al., 2011
Suzuki and Isemura, 2001
Mineva et al., 2013
Shankar et al., 2008



**These cellular processes
are essential for
brain development**

**EGCG - preclinical studies to
prevent developmental FAS
adverse effects**

Long et al., 2010
Tiwari et al., 2010

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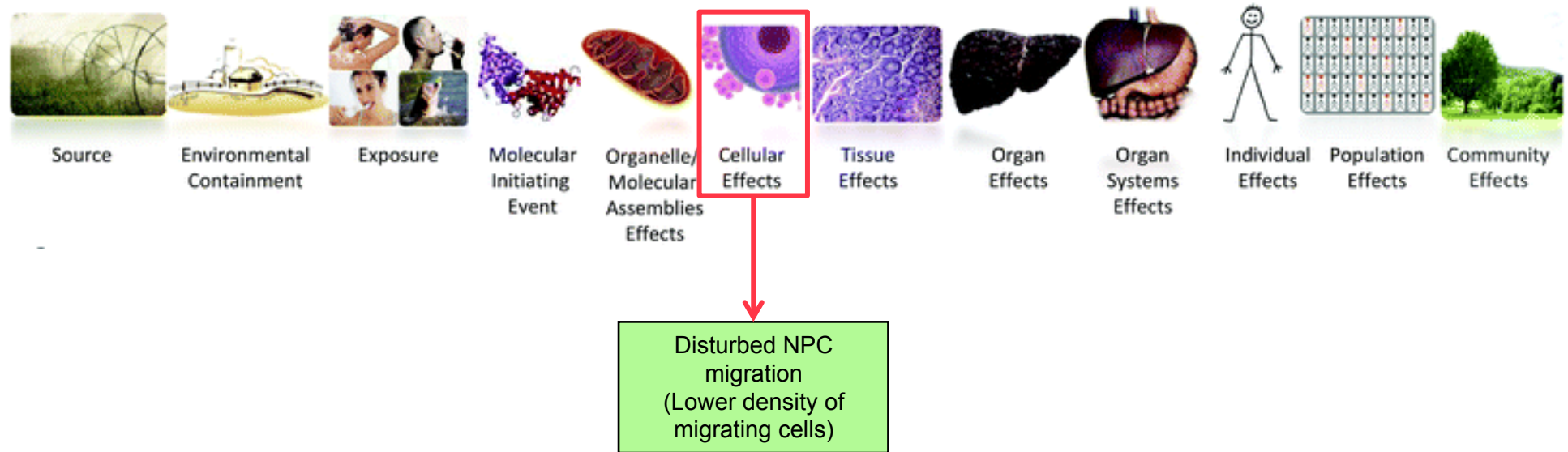
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EGCG's potential to cause neurodevelopmental toxicity is not known.

EGCG disturbs migration of rat and human NPCs *in vitro*

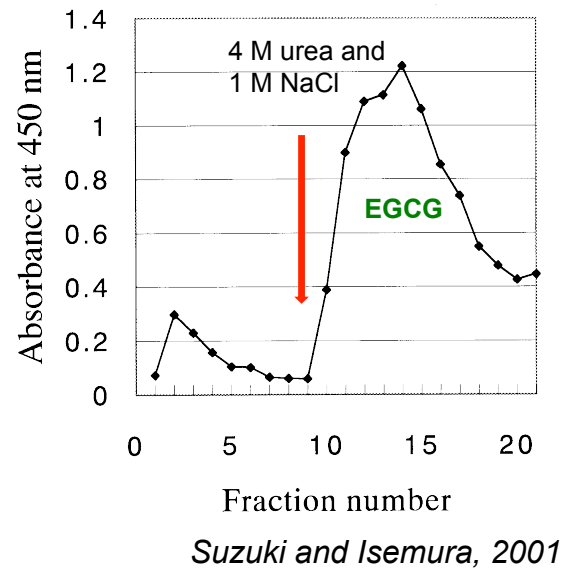
unpublished data

Putative AOP on DNT caused by disrupted laminin- β 1-integrin interaction.



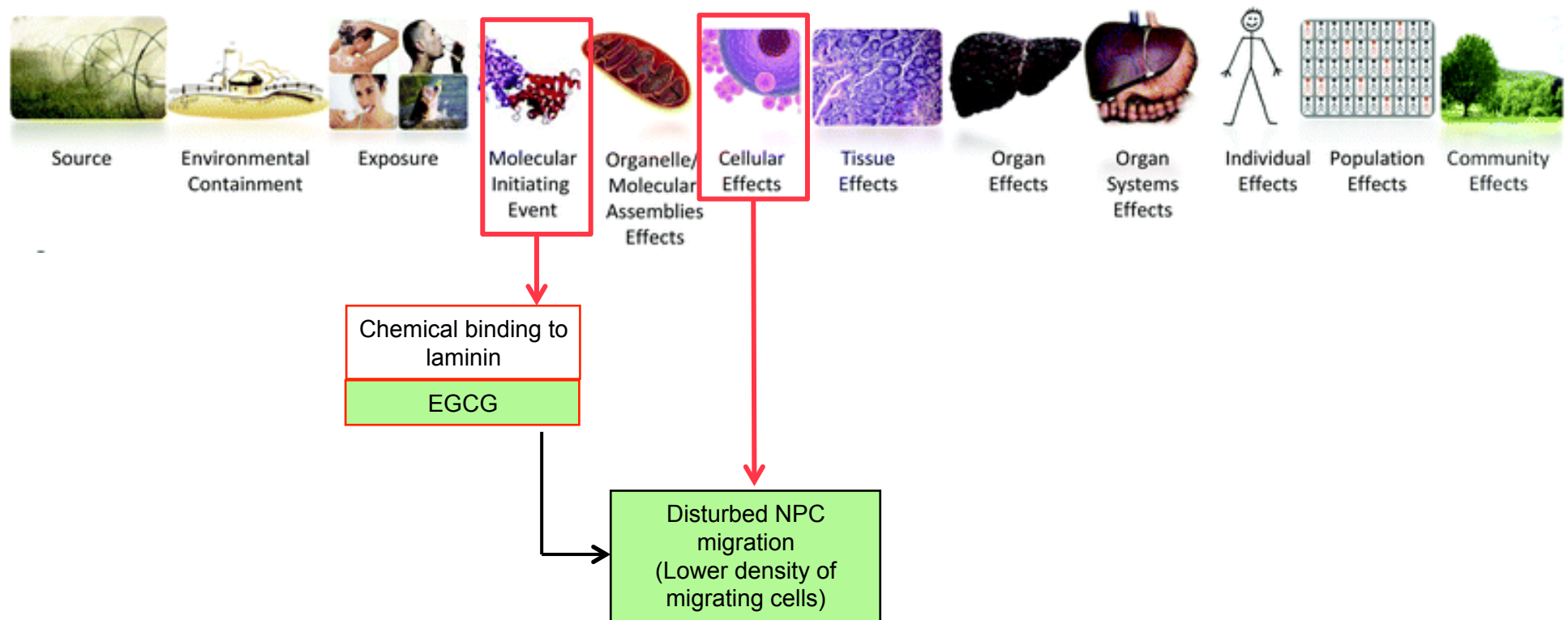
EGCG disturbs migration of rat and human NPCs in vitro ...

Affinity chromatography

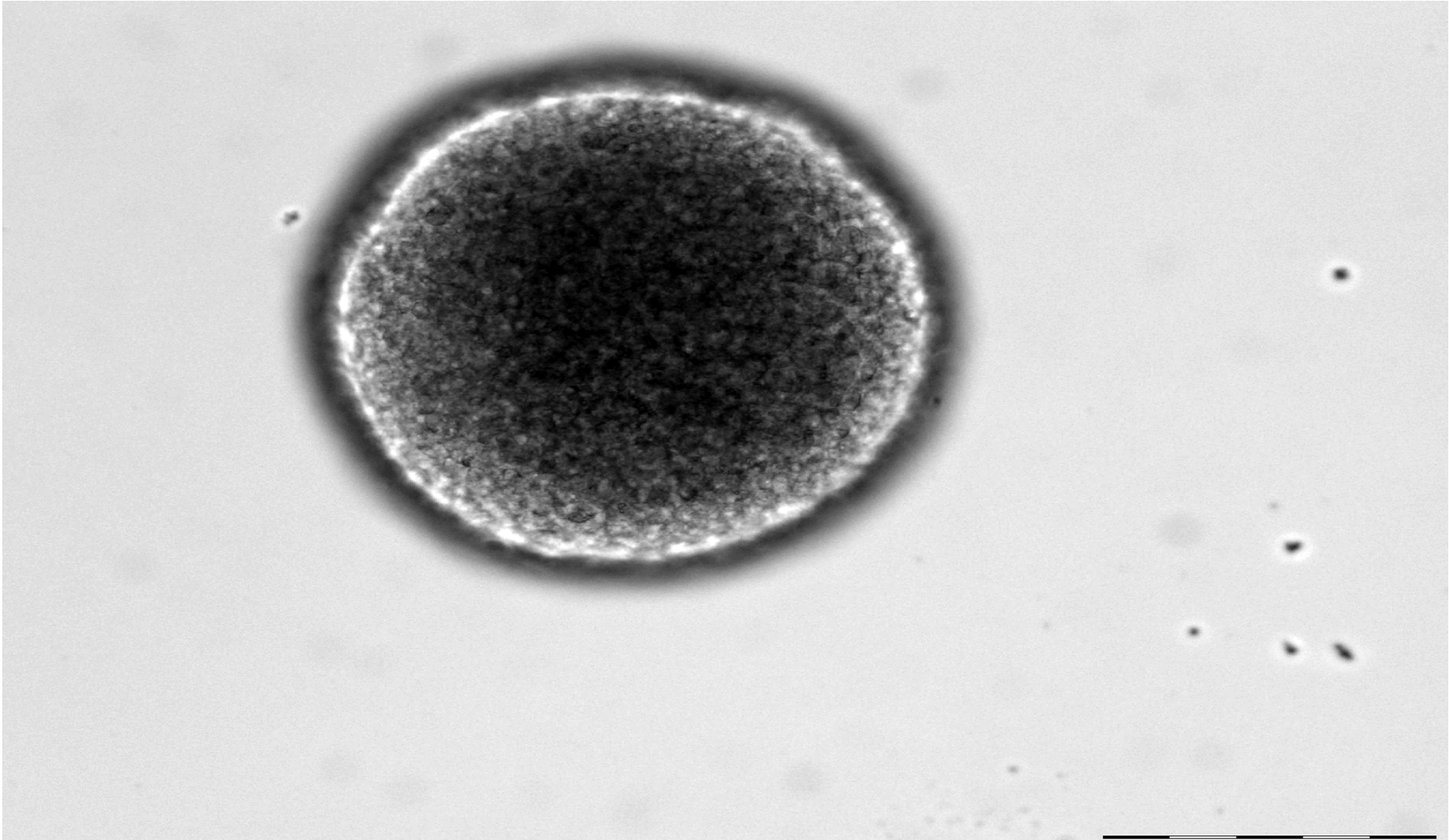


unpublished data

Putative AOP on DNT caused by disrupted laminin- β 1-integrin interaction.



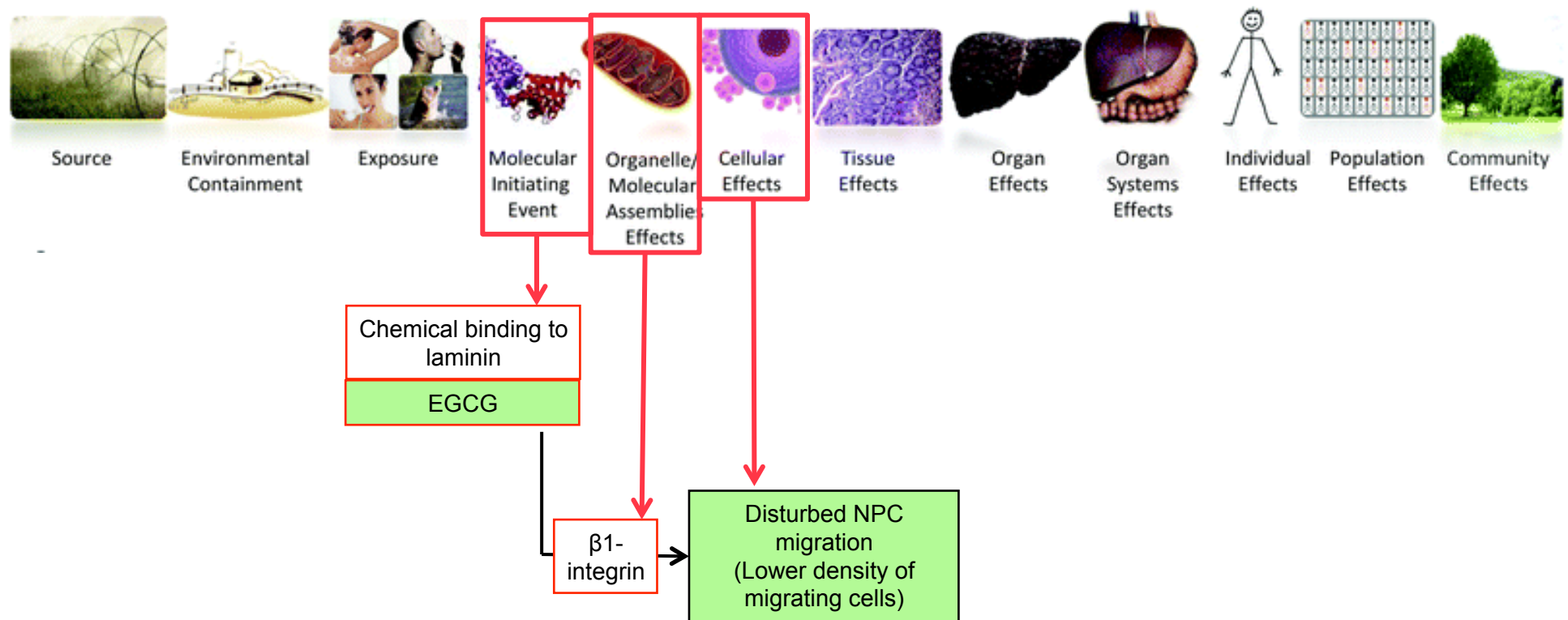
from
Ankley et al., 2009



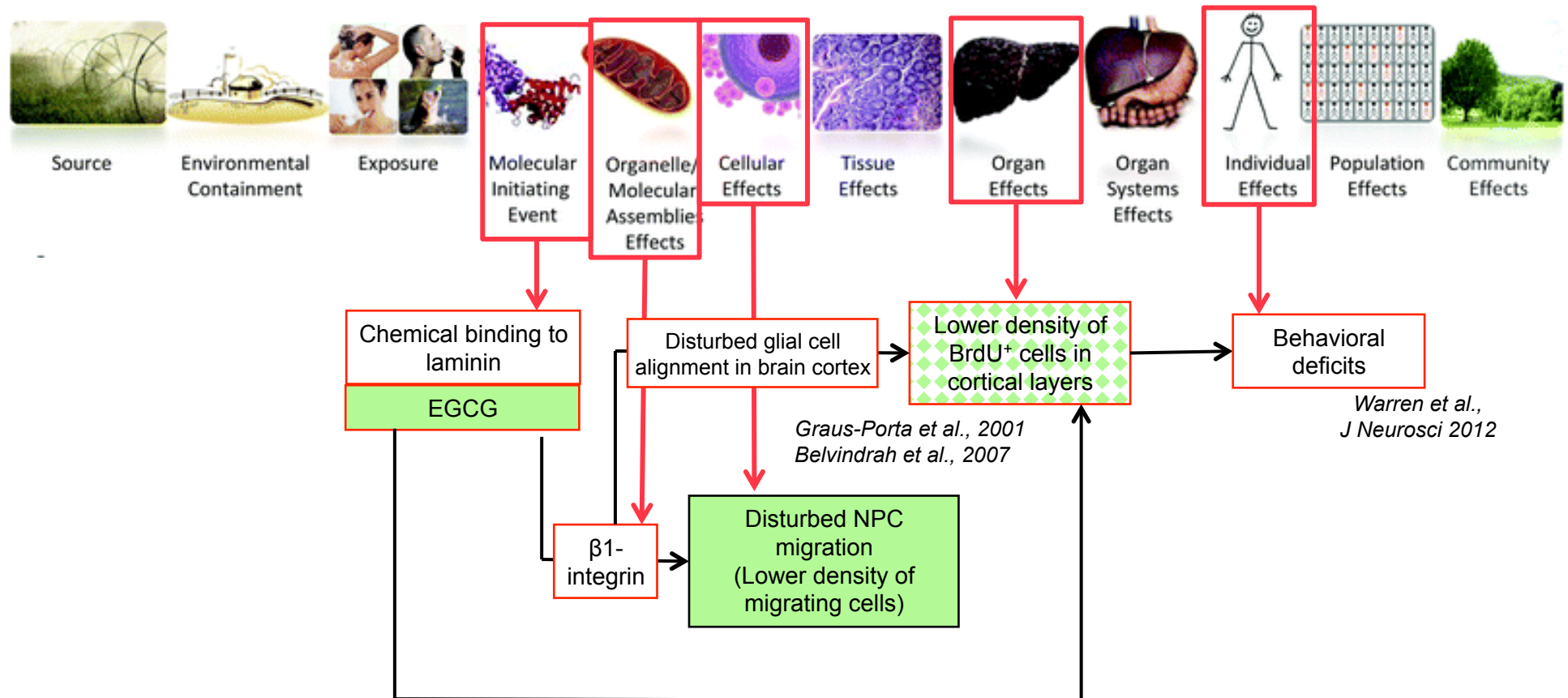
EGCG disturbs adhesion of rat and human NPCs in vitro ...

unpublished data

Putative AOP on DNT caused by disrupted laminin- β 1-integrin interaction.



Putative AOP on DNT caused by disrupted laminin- β 1-integrin interaction.



from
Ankley et al., 2009

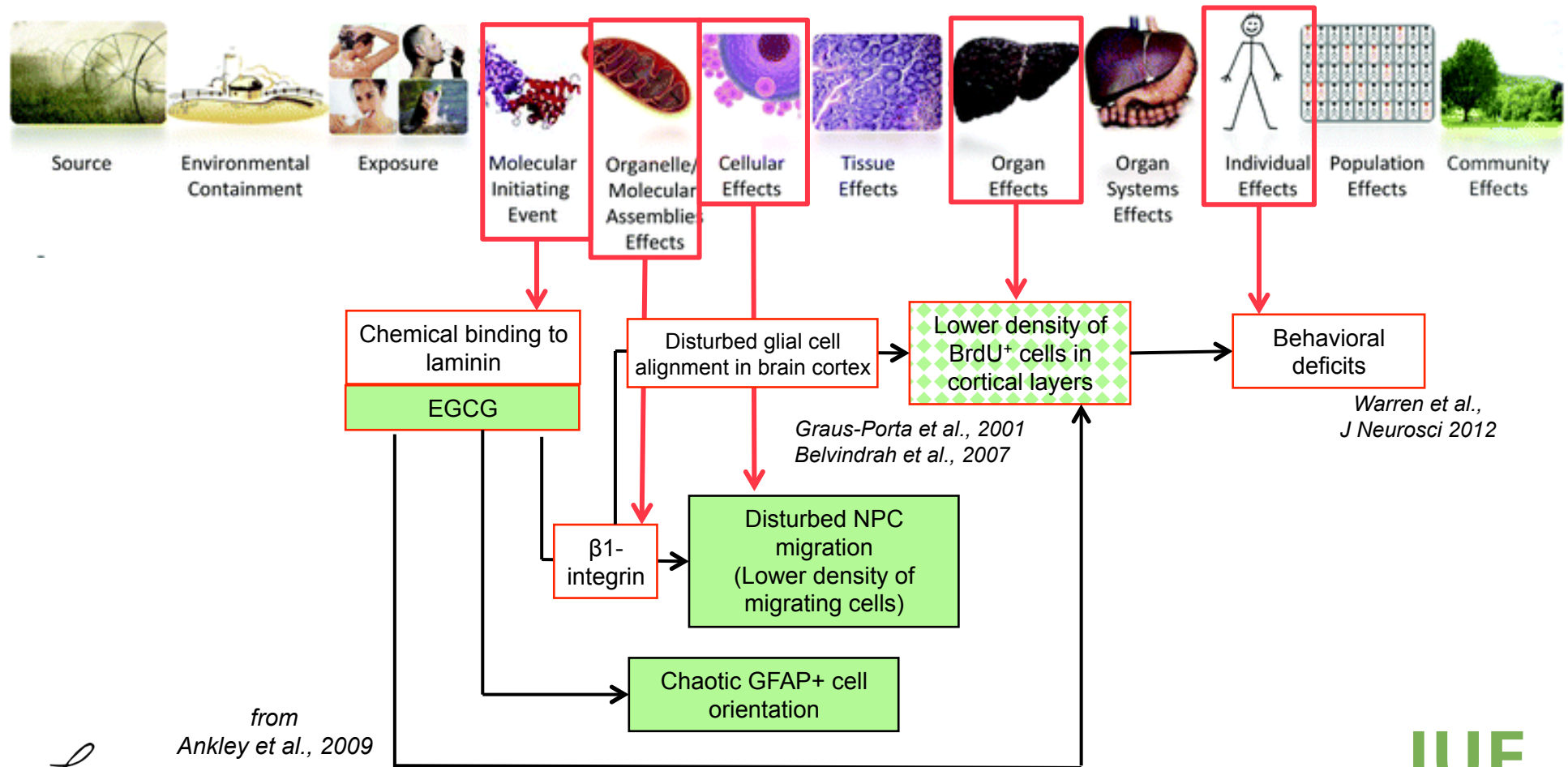
EGCG disturbs alignment of radial glia cells

unpublished data

EGCG causes secondary neuronal loss

unpublished data

Putative AOP on DNT caused by disrupted laminin- β 1-integrin interaction.

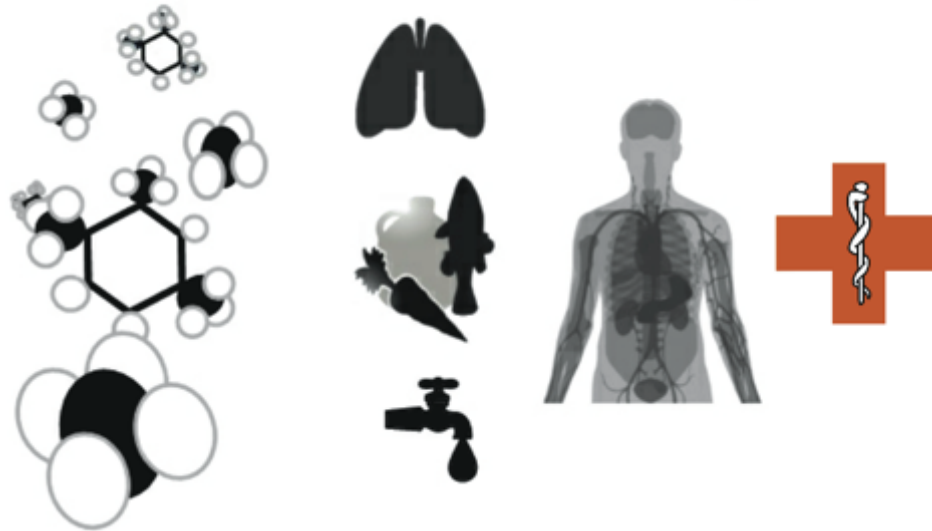


Exposure

Source ➤ Emissions ➤ Concentration ➤ Exposure ➤ Dose ➤ Health effects



www.pinterest.com



Modified after Shonkoff et al. 2014

Putative, qualitative ➔ quantitative AOP

Pharmacokinetic assessment of EGCG food supplement intake

Pharmacokinetics



EGCG oral supplements available via the Internet:

$$\begin{array}{l} \text{Concentration} \\ 0.1 \text{ g EGCG/mL} \end{array} \times \begin{array}{c} \text{Dose} \\ \text{3 spoons} \end{array} = 3 \text{ g}$$

Ullmann et al., 2003
Shanafelt et al., 2009
Chu et al., 2007
Suganuma et al., 1998
Zini et al., 2006

From EGCG **pharmacokinetics in humans:**

Dose	C_{\max} plasma
1.6 and 2 g EGCG/d	7.4 and 8.7 μM

Rev. in Barenys et al. 2015

Considering **pregnant women**, extrapolating from:

Dose	C_{\max} maternal plasma
3 g EGCG/d	11.9 to 22.2 μM

From EGCG **pharmacokinetics in rats:**

C_{\max} maternal plasma	C_{\max} fetal brain
0.6 μM	0.0761 μM

Assuming similar pharmacokinetics in pregnant rats and humans, which is supported by comparable EGCG kinetics in non-pregnant individuals

Dose	C_{\max} maternal plasma	C_{\max} fetal brain
3 g EGCG/d	11.9 to 22.2 μM	1 to 3 μM

Why do we need the AOP concept?

Paraquat is not a neurotoxicity hazard

The neurotoxic potential of paraquat has been extensively studied in laboratory animals. No clinical signs of neurotoxicity or consistent neuropathological changes have been reported following long-term exposures of dietary administration of paraquat to rodents or dogs in regulatory-compliant studies.

Source:

US Environmental Protection Agency, Office of Prevention, Pesticides and toxic substances. 2006. Memorandum: Risk Assessment. Subject: Paraquat Dichloride: Human Health Risk Assessment for Proposed Uses on Ginger and Okra and Amended Uses on Soybeans, Wheat, Cotton, Cucurbits, Onions, and Tanier. PC Code: 061601, Petition Nos: 2F6433, 3E6764, 1E6223, 1E6332, 3E6763, and 1E6319, DP Barcode: D328653.



Ntzani EE, Chondrogiorgi M,
Ntritsos G, Evangelou E, Tzoulaki I

EFSA supporting publication 2013:EN-497

EXTERNAL SCIENTIFIC REPORT

Literature review on epidemiological studies linking exposure to pesticides and health effects¹

Evangelia E Ntzani, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I

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Association between pesticide exposure and PD



Epidemiological
Studies

Parkinson's Disease



Ntzani EE, Chondrogiorgi M, Ntritsos G, Evangelou E, Tzoulaki I, 2013. Literature review on epidemiological studies **linking exposure to pesticides** and health effects. EFSA supporting publication 2013:EN-497, 159 pp.

MANDATE 1

- **Scientific opinion**
 - **2014 – 2016:** The working group is expected to:
 - Develop a prototype for assessing risk factors for Parkinson's disease/childhood leukaemia using the principles established for adverse outcome pathways (OECD, 2013)
 - Evaluate if, how and to what extent the experimental toxicity studies on mechanisms of toxicity cover effects and modes of action that are relevant for Parkinson's disease and childhood leukaemia
 - Address eventual data gaps and potential weaknesses in the current regulatory dossiers in supporting the hazard assessment
- **Call for tender** (OC/EFSA/PRAS/2014/01)
 - **2014** Systematic literature review on Parkinson's disease and childhood leukaemia and mode of actions for pesticides
- **Public consultation**
 - **2016** - Public consultation on the scientific opinion will be available on EFSA's website



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