

# The QSAR Toolbox to support the genotoxicity assessment of chemicals: the experience of the Istituto Superiore di Sanità (Italy)

**Cecilia Bossa**

ISS - Environment and Health Department

ISS is the technical and scientific body of the Italian Ministry of Health and the main centre for research, control and technical-scientific advice on public health in Italy

The activity of the **Environment and Health Department** is focused on multidisciplinary research which includes the identification and characterization of environmental and social risk factors, the study of their effects on health, and risk assessment



*The views and opinions expressed in this presentation are those of the author and do not necessarily reflect the official policy or position of ISS*

***in silico* Toxicology Unit** founded by Romualdo Benigni

Chiara Laura Battistelli, Cecilia Bossa, Alessandro Giuliani, Olga Tcheremenskaia

### **Development and application of computational methods in the assessment of toxicological risk**

- ✓ Implementation, evaluation and development of structure-activity relationships-based methodologies (e.g., (Q)SAR, read across, grouping, AOP and IATA approaches) to support chemicals (including nanomaterials) risk assessment.
- ✓ Study of the mechanism of action of genotoxic and carcinogenic chemicals; coding of structural determinants for chemicals toxicity (genotoxicity/carcinogenicity).
- ✓ Development of statistical and Systems-Biology models
- ✓ Analysis and standardization of experimental data, creation of chemical relational databases on toxicological endpoints.
- ✓ The group is actively committed in promoting the QSAR Toolbox (*e.g.*, through the donation of ISS databases and profilers and participation in the OECD QSAR Toolbox Management Group)



## Institutional tasks

- ✓ REACH related activity
- ✓ National remediation sites related activity



## Research activity

- ✓ Applicability of in silico models for predicting the genotoxicity of pesticides
- ✓ Japan DGM/NIHS 1<sup>st</sup> Ames/QSAR international collaborative project

## REACH related activity

ISS supports the Member State Competent Authority in substance evaluation (Community Rolling Action Plan - CoRAP) and dossier evaluation

→ Evaluation of QSARs, Read across and Grouping approaches (genotoxicity/carcinogenicity)

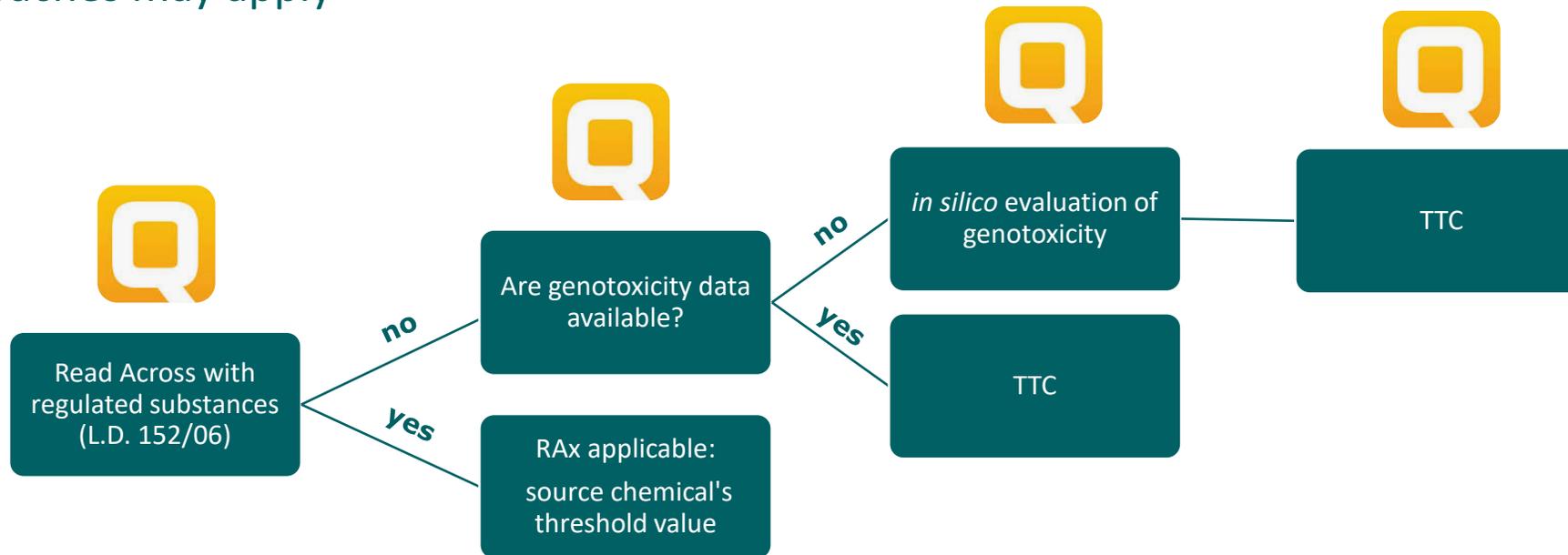
- Structure inspection, chemical identity verification (including CAS-SMILES relation)
- Experimental data availability
- Toxicological profiling (*in silico*)
- Insights on the mechanism of action
- Search for analogues
- Verification of RAx elements, Chemical Category consistency, QSAR results
- Data matrix generation, in tabular form (working basis)



## National remediation sites related activity

ISS is asked to express opinions on the definition of the threshold concentration of contamination (substances not included in the Legislative Decree 152/06).

In the presence of a non-regulated chemical, lacking sufficient toxicological information, *in silico* approaches may apply



## Institutional tasks

- ✓ REACH related activity
- ✓ National remediation sites related activity



## Research activity

- ✓ Applicability of in silico models for predicting the genotoxicity of pesticides
- ✓ Japan DGM/NIHS 1<sup>st</sup> Ames/QSAR international collaborative project

# Applicability of *in silico* models for predicting the genotoxicity of pesticides (EFSA funded project - OC/EFSA/PRAS/2016/01)

- Characterization of the structural changes resulting from metabolic or degradation processes of the active substances and evaluation of their impact on the genotoxicity potential

## EXTERNAL SCIENTIFIC REPORT

APPROVED: 12 March 2019  
doi:10.2903/sp.efsa.2019.EN-1598

### Evaluation of the applicability of existing (Q)SAR models for predicting the genotoxicity of pesticides and similarity analysis related with genotoxicity of pesticides for facilitating of grouping and read across

Romualdo Benigni<sup>a</sup>, Chiara Laura Battistelli<sup>a</sup>, Cecilia Bossa<sup>a</sup>, Alessandro Giuliani<sup>a</sup>, Elena Fioravanzo<sup>a</sup>, Arianna Bassan<sup>a</sup>, Mojca Fuat Gatnik<sup>a</sup>, James Rathman<sup>a</sup>, Chihae Yang<sup>a</sup> and Olga Tcheremenskaia<sup>a</sup>

Regulatory Toxicology and Pharmacology 114 (2020) 104658

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: [www.elsevier.com/locate/yrtph](http://www.elsevier.com/locate/yrtph)

Evaluation of the applicability of existing (Q)SAR models for predicting the genotoxicity of pesticides and similarity analysis related with genotoxicity of pesticides for facilitating of grouping and read across: An EFSA funded project

Romualdo Benigni<sup>a</sup>, Rositsa Serafimova<sup>b</sup>, Juan Manuel Parra Morte<sup>b</sup>, Chiara Laura Battistelli<sup>a</sup>, Cecilia Bossa<sup>a</sup>, Alessandro Giuliani<sup>a</sup>, Elena Fioravanzo<sup>a</sup>, Arianna Bassan<sup>a</sup>, Mojca Fuat Gatnik<sup>a</sup>, James Rathman<sup>a</sup>, Chihae Yang<sup>a</sup>, Aleksandra Mostrag-Szlichtyng<sup>c</sup>, Oliver Sacher<sup>c</sup>, Olga Tcheremenskaia<sup>a</sup>

## About Genotoxicity pesticides EFSA

About

Name  
Genotoxicity pesticides EFSA

Short Description

Genotoxicity EFSA pesticides database comprises genotoxicity data of 290 parent pesticides and their metabolites [1]. Data have been collected for different genotoxicity endpoints:

- Bacillus subtilis recombination assay
- Bacterial reverse mutation assay (e.g. Ames test)
- DNA damage and repair assay, unscheduled DNA synthesis in mammalian cells in vitro
  - Dominant lethal assay
  - Drosophila SLRL Test
  - in Vitro Gene Mutation Assay in Fungi
  - in Vitro Mammalian Cell Micronucleus Test
  - in Vitro Mammalian Cell Transformation Assay
  - in Vitro Mammalian Chromosome Aberration Test
  - in Vivo Mammalian Chromosome Aberration Test
  - Mammalian Cell Gene Mutation Assay
  - Mammalian erythrocyte micronucleus test
  - Mammalian Germ Cell Cytogenetic Assay
  - Mitotic Recombination in Saccharomyces Cerevisiae
  - Mouse Spot Test
  - Single cell gel electrophoresis (comet) assay
  - Sister Chromatid Exchange Assay in Mammalian Cells
  - SOS/umu test
  - Unscheduled DNA Synthesis
  - Yeast Cytogenetic Assay
  - other

#### References:

1. F. Metruccio, I. Castelli, C. Civitella, C. Galbusera, F. Galimberti, L. Tosti, A. Moretto, 2017. Compilation of a database, specific for the pesticide active substance and their metabolites, comprising the main genotoxicity endpoints. EFSA supporting publication 2017:EN-1229. 125 pp. doi:10.2903/sp.efsa.2017.EN-1229

Disclaimer

Donator(s)

European Food Safety Authority (EFSA)

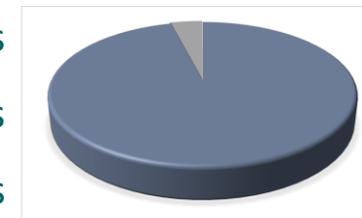


### *in vitro* bacterial reverse mutation assay

#### (Ames test)

Positive 4%  
Negative 96%

~ 900 chemicals  
1/3 active substances  
2/3 metabolites



# Analysis of the structural changes (in terms of functional groups) that occur in the transformation from the parent substance to the metabolites

- ✓ Focus on the metabolites which maintain the core structure of the parent  
→ similarity analysis

Similarity options

Measure

Tanimoto (Jaccard)

Dice

Kulczynski-2

Ochiai (Cosine)

Yule

Molecular features

Atom pairs

Topologic torsions

Atom centered fragments

Path

Cycles

PubChem features

Options

Calculation

Fingerprint

Hologram

Average by features

Combine all features

Atom characteristics

Atom type

Count H attached

Count heavy atoms attached

Hybridization

Incident pi-bonds

Valency

Charge

Cyclic

Formula

$c/0.5[(a+c)+(b+c)]$

Description

The atom-centered fragment is a topological sphere with center a selected atom and radius specified in **Any atom distance**. For aromatic carbon as a center of the sphere is assumed the aromatic system that contains this atom of concern.

Structure

Define

Example

A	B	C
2	2	10

Similarity = 83.333% Details

Default Help

OK Cancel



We selected 319 AS/metabolite pairs with similarity  $\geq 70\%$

# Analysis of the structural changes (in terms of functional groups) that occur in the transformation from the parent substance to the metabolites

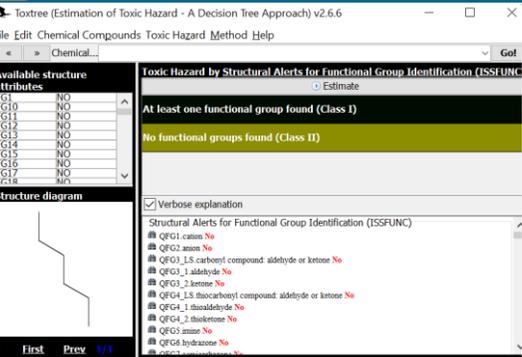
- ✓ Focus on the metabolites which maintain the core structure of the parent  
→ **similarity analysis**
- ✓ Characterize the structural features that change more frequently in the transformation from the parent to the metabolites  
→ **functional group analysis**

# Analysis of the structural changes (in terms of functional groups) that occur in the transformation from the parent substance to the metabolites

- ✓ Characterize the structural features that change more frequently in the transformation from the parent to the metabolites  
→ functional group analysis

Empiric

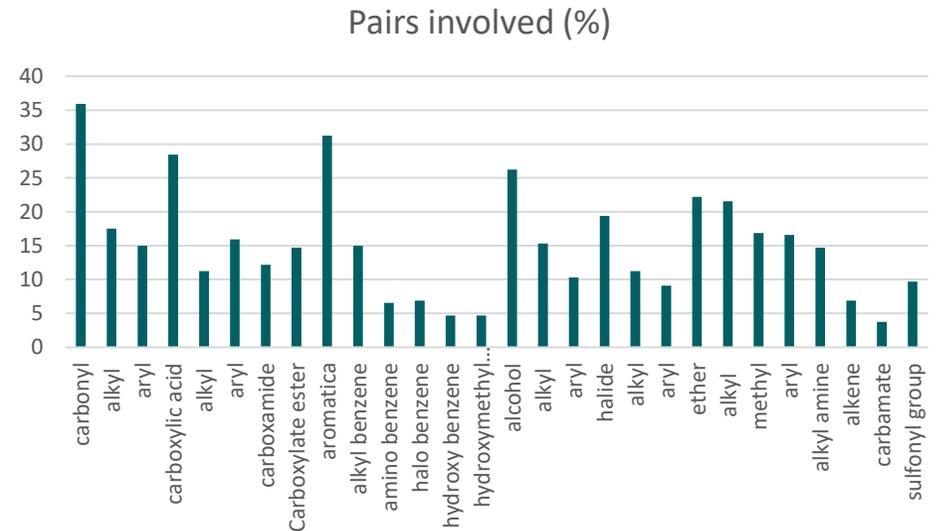
- Chemical elements
- Groups of elements
- Lipinski Rule Oasis
- Organic functional groups
- Organic functional groups (nested)
- Organic functional groups (US EPA)
- Organic functional groups, Norbert Haider (checkmol)
- Structure similarity
- Tautomers unstable



The screenshot shows the Toxtree software interface. The top panel displays a list of structure attributes with checkboxes. The main window shows the results of the ISSFUNC profiler, indicating that at least one functional group was found (Class I). A list of structural alerts is provided, such as 'QF01 oxime No', 'QF02 amine No', and 'QF03 1,5 carbonyl compound: aldehyde or ketone No'.

Toolbox profilers

Toxtree – ISSFUNC profiler

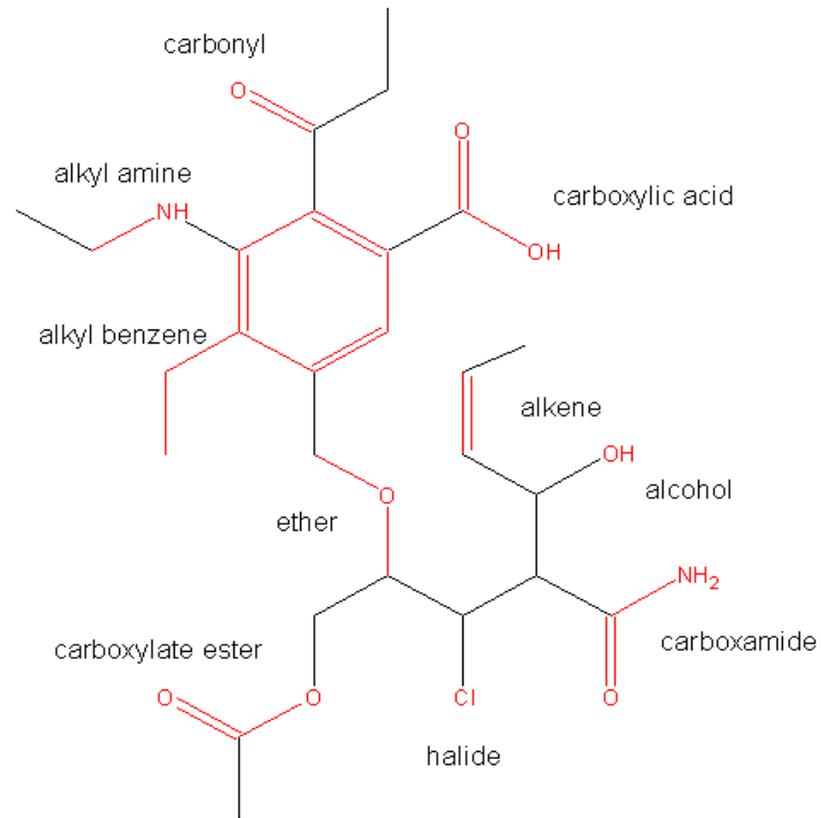


# Analysis of the structural changes (in terms of functional groups) that occur in the transformation from the parent substance to the metabolites

- ✓ Focus on the metabolites which maintain the core structure of the parent  
→ **similarity analysis**
- ✓ Characterize the structural features that change more frequently in the transformation from the parent to the metabolites  
→ **functional group analysis**
- ✓ Evaluate the impact of the structural changes on the mutagenicity potential  
→ **structure-activity relationships analysis**

# Analysis of the structural changes (in terms of functional groups) that occur in the transformation from the parent substance to the metabolites

- ✓ Evaluate the impact of the structural changes on the mutagenicity potential  
→ **structure-activity relationships analysis**



Non-toxifying structural changes in Parent / Metabolite pairs with high similarity

# Improvement of QSAR profiler for predicting Ames mutagenicity

## 1<sup>st</sup> Ames/QSAR international collaborative project

Mutagenesis, 2019, 34, 3–16  
doi:10.1093/mutage/gey031

Original Manuscript

OXFORD

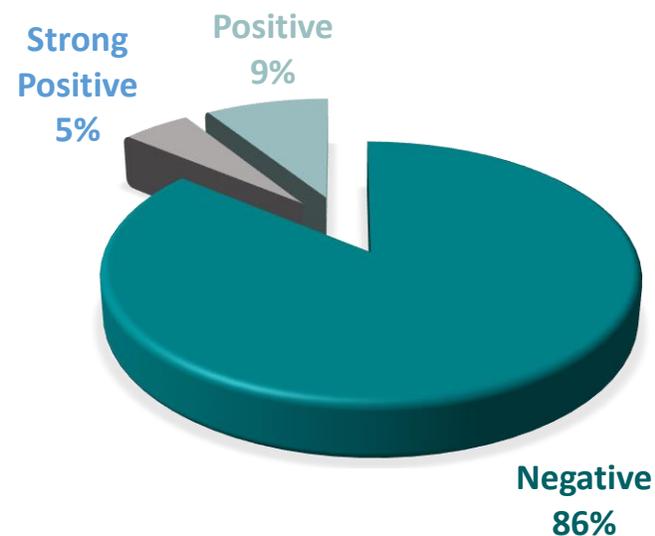
Original Manuscript

### Improvement of quantitative structure–activity relationship (QSAR) tools for predicting Ames mutagenicity: outcomes of the Ames/QSAR International Challenge Project

Masamitsu Honma<sup>\*</sup>, Airi Kitazawa, Alex Cayley<sup>1</sup>, Richard V. Williams<sup>1</sup>, Chris Barber<sup>1</sup>, Thierry Hanser<sup>1</sup>, Roustem Saiakhov<sup>2</sup>, Suman Chakravarti<sup>2</sup>, Glenn J. Myatt<sup>3</sup>, Kevin P. Cross<sup>3</sup>, Emilio Benfenati<sup>4</sup>, Giuseppa Raitano<sup>4</sup>, Ovanes Mekenyan<sup>5</sup>, Petko Petkov<sup>5</sup>, Cecilia Bossa<sup>6</sup>, Romualdo Benigni<sup>6,7</sup>, Chiara Laura Battistelli<sup>9</sup>, Alessandro Giuliani<sup>6</sup>, Olga Tcheremenskaia<sup>6</sup>, Christine DeMeo<sup>8</sup>, Ulf Norinder<sup>8,10</sup>, Hiromi Koga<sup>11</sup>, Ciloy Jose<sup>11</sup>, Nina Jeliazkova<sup>12</sup>, Nikolay Kochev<sup>12,13</sup>, Vesselina Paskaleva<sup>13</sup>, Chihae Yang<sup>14</sup>, Pankaj R. Daga<sup>15</sup>, Robert D. Clark<sup>15</sup> and James Rathman<sup>14,16</sup>

- Promoted by the Japan National Institute of Health Sciences (DGM/NIHS), aimed at improving the reliability and applicability of QSAR models for predicting Ames mutagenicity, by the analysis of new Ames test results

More than 12,000 chemicals tested according to the OECD TG471 guideline



# Improvement of QSAR profiler for predicting Ames mutagenicity

→ in vitro mutagenicity (Ames test) alerts by ISS – Toxtree /QSAR Toolbox profiler

- Chemical structure inspection
- Functional groups analysis
- Toxicological profiling (*in silico*)
- Insights on the mechanism of action



QSAR Toolbox 4.5 [Document 1]

Profiling methods: 9 Selected

- Skin irritation/corrosion Exclusion rules
- Skin Irritation/corrosion Inclusion rules
- Empiric
  - Chemical elements
  - Groups of elements
  - Lipinski Rule Oasis
  - Organic functional groups
  - Organic functional groups (nested)
  - Organic functional groups (US EPA)
  - Organic functional groups, Norbert Ha...
- Toxicological
  - Repeated dose (HESS)
- Custom
  - Example Prioritization Scheme (PBT)
  - New Profiler
  - New Profiler 2

Structure	36	37	38	39	40
Structure					
Ecotoxicological Information					
Human Health Hazards					
Profiling					
General Mechanistic					
DNA binding by OASIS	AN2	No alert found	No alert found	Radical	No alert found
DNA binding by OECD	Michael addition	No alert found	No alert found	SN1	SN1
Endpoint Specific					
DNA alerts for AMES, CA and MNT by...	AN2	No alert found	No alert found	Radical	No alert found
in vitro mutagenicity (Ames test) alert...	Quinones	No alert found	No alert found	Hydrazine	No alert found
Oncologic Primary Classification	Not classified	Halogenated Aromatic...	Not classified	Aromatic Amine Type...	Not classified
Empiric					
Organic functional groups	Alkene moiety	Alkyl (hetero)arenes	Aryl	Aryl	Aliphatic amine, tertiary
Organic functional groups (nested)	Diketone	Alkyl (hetero)arenes	Aryl	Furan	Aliphatic amine, tertiary
Organic functional groups (US EPA)	Aliphatic Carbon [-CH...	Aliphatic Carbon [-CH...	Acid, aromatic attach [...	1,2-Oxaza compounds...	Acid, aliphatic attach [...
Organic functional groups, Norbert Ha...	Aromatic compound	Aromatic compound	Aromatic compound	Aromatic compound	Amine

## QSAR Toolbox Custom Profile functionality

New Profiler 2 (Custom) - Profiling Scheme Browser

Categories: New Profiler 2, Alert 1

[1] Category 1

Query details

[1] Structure Query | Metabolism

Contents

- Add Query
- Add Mask
- Remove

Complex search options

- Exact connectivity
- Ignore stereo information
- Exact match

Queries execution mode: All

Mapping

- Unique mappings
- Max maps: 1000

SMARTS: c1cc(cc(c1)O)NJC

View mode: Facade | Navigation mode: Cascade

Left click on any marked atom to explore

Cecilia Bossa

# QSAR Toolbox related publications

- Benigni R (2021) Regul. Toxicol. Pharmacol. 126: 105042. <https://doi.org/10.1016/j.yrtph.2021.105042>
- Benigni R, Serafimova R, Parra Morte JM, Battistelli CL, Bossa C, Giuliani A, Fioravanzo E, Bassan A, Fuart Gatnik M, Rathman J, Yang C, Mostrag-Szlichtyng A, Sacher O, Tcheremenskaia O. (2020) Regul. Toxicol. Pharmacol. 114: 104658 <https://doi.org/10.1016/j.yrtph.2020.104658>
- Honma M, Kitazawa A, Cayley A, Williams RV, Barber C, Hanser T, Saiakhov R, Chakravarti S, Myatt GJ, Cross KP, Benfenati E, Raitano G, Mekenyan O, Petkov P, Bossa C, Benigni R, Battistelli CL, Giuliani A, Tcheremenskaia O, DeMeo C, Norinder U Koga H, Jose C, Jeliaskova N, Kochev N, Paskaleva V, Yang C, Daga PR, Clark RD, Rathman J. (2019) Mutagenesis 34(1):3-16. <https://doi.org/10.1093/mutage/gy031>
- Benigni R, Battistelli CL, Bossa C, Giuliani A, Fioravanzo E, Bassan A, Fuart Gatnik M, Rathman J, Yang C, Tcheremenskaia O. (2019) EFSA Supporting publications <https://doi.org/10.2903/sp.efsa.2019.EN-1598>
- Tcheremenskaia O, Battistelli CL, Giuliani A, Benigni R, Bossa C. (2019) Comput toxicol, 11:91-100. <https://doi.org/10.1016/j.comtox.2019.03.005>
- Bossa C, Benigni R, Tcheremenskaia O, Battistelli CL. (2018) Computational Toxicology. Methods in Molecular Biology, vol 1800. Humana Press, New York, NY. [https://doi.org/10.1007/978-1-4939-7899-1\\_20](https://doi.org/10.1007/978-1-4939-7899-1_20)
- Benigni R, Battistelli CL, Bossa C, Tcheremenskaia O, Giuliani A. (2017) Regul. Toxicol. Pharmacol. 86:18-24. <https://doi.org/10.1016/j.yrtph.2017.02.013>
- Benigni R, Bossa C, Tcheremenskaia O. (2016) Regul. Toxicol. Pharmacol., 78:45-52. <https://doi.org/10.1016/j.yrtph.2016.04.003>
- Benigni R, Bossa C, Tcheremenskaia O, Battistelli CL, Giuliani A. (2015) Mutat Res Genet Toxicol Environ Mutagen 779:35-38. <https://doi.org/10.1016/j.mrgentox.2015.02.001>
- Benigni R, Bossa C and Tcheremenskaia O. (2013) Chem. Rev. 113, 5: 2940–2957 <https://doi.org/10.1021/cr300206t>
- Benigni R, Battistelli CL, Bossa C, Tcheremenskaia O and Crettaz P. (2013) Mutagenesis 28(4):401-9 <https://doi.org/10.1093/mutage/get016>
- Benigni R, Bossa C and Tcheremenskaia O (2013) Mutagenesis 28(1): 107-116. <https://doi.org/10.1093/mutage/ges059>
- Benigni R, Battistelli CL, Bossa C, Colafranceschi M, Tcheremenskaia O. (2013) Mutagenicity, Carcinogenicity, and Other End points. In: Reisfeld B., Mayeno A. (eds) Computational Toxicology. Methods in Molecular Biology (Methods and Protocols), vol 930. Humana Press, Totowa, NJ. [https://doi.org/10.1007/978-1-62703-059-5\\_4](https://doi.org/10.1007/978-1-62703-059-5_4)
- Benigni R, Bossa C, Battistelli CL, Tcheremenskaia O. (2013) Mutat Res Genet Toxicol Environ Mutagen 12;758(1-2):56-61. <https://doi.org/10.1016/j.mrgentox.2013.09.006>
- Benigni R, Bossa C, Tcheremenskaia O, Battistelli CL and Crettaz P. (2012) Mutagenesis 27(1): 87-92. <https://doi.org/10.1093/mutage/ger064>
- Benigni R and Bossa C. (2011) Chem. Rev. 111:2507-2536. <https://doi.org/10.1021/cr100222q>
- Benigni R, Bossa C and Worth A. (2010) Mutagenesis 25(4): 335-341. <https://doi.org/10.1093/mutage/geq010>

# Acknowledgements

## **ISS *in silico* toxicology unit**

Dr Chiara L. Battistelli

Dr Alessandro Giuliani

Dr Olga Tcheremenskaia

**Dr Romualdo Benigni (Alpha-PreTox)**

**Thank you for your attention!**