Overview of NATO SfP project Ecotoxicity of metal and metal oxide nanoparticles: experimental study and modelling

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Nano WG Presentation

12 September 2013

Ecotoxicity of metal and metal oxide nanoparticles: experimental study and modelling

Project number: 984401

NPD Co-Director – Prof. PEIJNENBURG Willie,
University of Leiden, Leiden, The Netherlands

PPD Co-Director – Prof. KUSTOV Leonid,
N.D. Zelinsky institute of Organic Chemistry,
Russian Academy of Sciences, Moscow, Russia

Other Co-director – Dr. Larisa Metelitsa, Institute of Bioorganic Chemistry and Petrochemistry, Ukrainian Academy of Sciences, Kyiv, Ukraine

End-user(s) of the Project results:

- JSC "Concern Nanoindustry", Moscow, Russia; Prof. M. ANANYAN
- eADMET GmbH, Germany, Munich, Dr. I.V. Tetko

NATO The Science for Peace and Security Programme

Facilitate mutually beneficial cooperation on issues of common interest, including international efforts to meet emerging security challenges

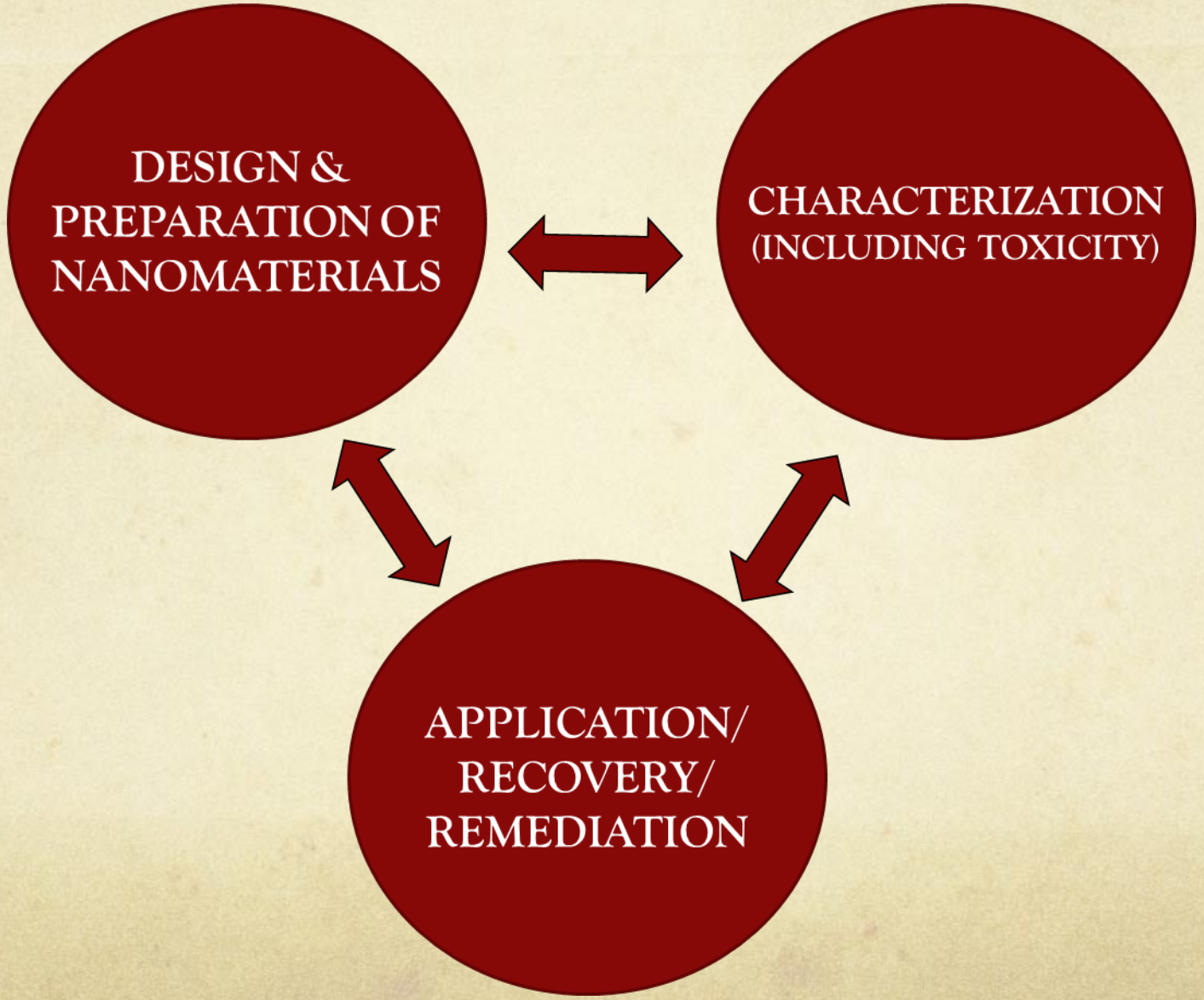
✓ **Environmental Security**

- Security issues arising from key environmental and resource constraints, including health risks, climate change, water scarcity and increasing energy needs, which have the potential to significantly affect NATO's planning and operations;
- Disaster forecast and prevention of natural catastrophes;
- Defence-related environmental issues.

SPS Key Priorities are based on NATO's Strategic Concept as agreed by Allies in Lisbon in November 2010 and the Strategic Objectives of NATO's Partner Relations as agreed in Berlin in April 2011, without any indication of priority ranking.

http://www.nato.int/science

SCOPE OF ONGOING ACTIVITIES



UNIVERSITY OF LEIDEN
Center Environmental Sciences

Universiteit Leiden

Dept. Conservation Biology: Biodiversity and (natural) stressors

- Impact of landscape factors on biodiversity
- Impact of invasive species on biodiversity
- Biodiversity in urban environments/green infractructures
- **Impact of chemicals on biodiversity**

KEY STAFF:

- Prof. Dr. Geert de Snoo (Head)
- Prof. Dr. Willie Peijnenburg (Team Leader)
- Dr. Martina Vijver (Senior Researcher)
- (+ Support staff, 6 PhD students, 4 young researchers)

Role in the project: Management and dissemination, elaboration of the algorithms and predictability of the ecotoxicity of nanomaterials, daphnia testing and assessment of some physicochemical properties (zeta potential and size distribution), organization of a workshop for the potential end-users, use of the NATO tools (NATO website, NATO Internet TV, video products, articles for NATO reviews) for dissemination of project results.

Institute of Organic Chemistry

ONGOING RESEARCH: Synthesis of NPs (metals, metal oxides, QDs), Physcicochemical characterization of NPs
-Nanotoxicity studies (algae, flat warms, daphnids, mussels), Application of NPs (catalysis, remediation, electronic materials, OLEDs)

KEY STAFF:

- Prof. Leonid Kustov (Head)
- Dr. Elena Finashina (Deputy)
- Dr. Olga Kirichenko (Senior Researcher)
- Dr. Olga Tkachenko (Senior Researcher)
- (+ 5 PhD students, 4 young researchers)

Role in the project: collection of data on available toxicity from literature for NANECO database, synthesis of free-standing and supported nanomaterials, characterization of nanomaterials by spectroscopic methods, including DRIFTS and XPS, adsorption studies, SEM/TEM, EXAFS/XANES, evaluation of the toxicity of nanomaterials using daphnids and mussels, development of project website.

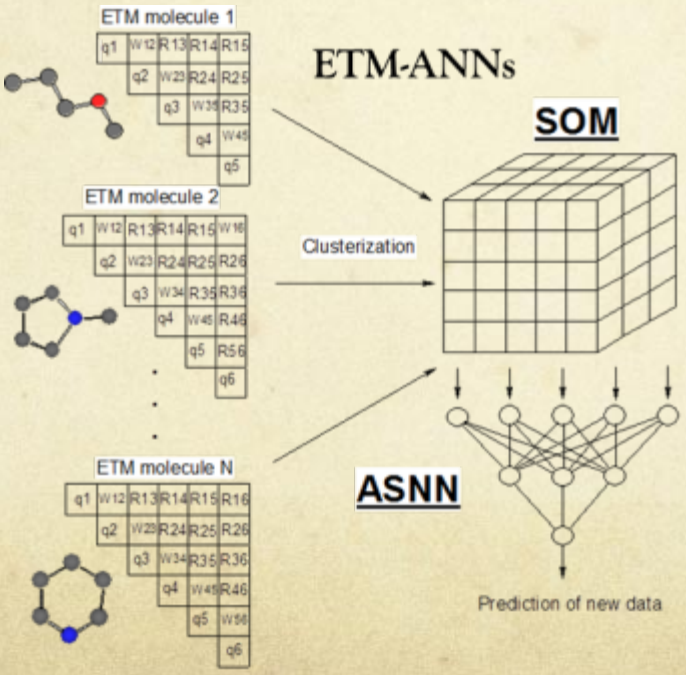
Institute of Bioorganic Chemistry and Petrochemistry

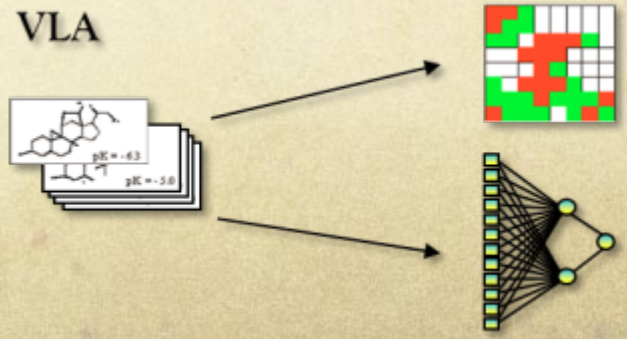
National Academy of Science of Ukraine

- Strongly involved in environmental protection policy in the Ukraine
- Development of new pattern recognition algorithms for structure-activity studies
- Predictive ADME and Toxicity model development
- Current studies are toward development of methods to predict NP toxicity

Role in the project: collection of data for NANECO database, QSAR models at the On-Line Chemical Modelling Environment (OCHEM - http://ochem.eu) site, in silico QSAR model development using experimental data, structural information and composition of NPs.

Dr. L. Metelitsa





eADMET GmbH

eADMET
THE REFERENCE IN CHEMOINFORMATICS

- Expertise in modelling of physico-chemical and biological properties
- Access to variety of machine learning algorithms and descriptors
- Database of more than 1M records for QSAR modelling
- Support of nanomaterials

END USERS

- **JSC Concern Nanoindustry, Russia**
  - Production of Ag NP (AgBion-1, AgBion-2 products) and TiO2 NP,
  - Co-funding of the extension of the project
  - Use of the concepts to other groups of nanomaterials
  - Use of the methods of nanotoxicity evaluation for the commercial products.

- **eADMET GmbH, Germany**
  - Development of software for prediction of biological and physico-chemical properties of chemicals.
  - Development of OCHEM platform to create NANECO database as the main portal.
  - Commercialization and distribution of the NANECO database and modeling algorithms developed within the project after its termination.
  - Participation in the research activities with its own budget.

Main Objectives

- Development of the NANECO database combining available literature and own data on the toxicity and NP-specific metrics affecting the fate and toxicity of nanomaterials.*
- Elaboration of algorithms to link observed ecotoxicity profiles with the measured and calculated properties of inorganic NP and their chemical nature.*
- Development of guidelines for the potential end-users for the production of safe (less hazardous) nanomaterials.

*The database and software is expected to be licensed at the end of the project by eADMET GmbH.

Intermediate Objectives

- Statistical experimental design
- Targeted design and synthesis of free-standing and supported metal and metal oxide NP with controlled particle size and morphology and physicochemical characterization of NPs by spectroscopic methods to derive descriptors in further modelling studies.
- Revealing dependencies between the toxicity of MPs towards aquatic organisms and the geometric characteristics of NPs determining ecotoxicity.

STEPS TOWARDS A PREDICTION ALGORITHM

1. **Collect the database on NP toxicity**

2. **Generate new data on NP toxicity**
   a) statistical experimental design
   b) synthesis of free standing and supported NPs: Δ particle size, morphology, crystalline structure, etc.
   c) phys.chem. characterisation of NPs - various types of spectroscopy
   d) assessment of toxicity profile

3. **Develop QSAR-type of the algorithm**: link toxicity profile to NP characteristics (descriptors) affecting toxicity (size, shape, morphology, band gaps, etc.) based on NP clusters

METHODOLOGY-1

Experimental work:

- Rational and systematic **synthesis of nanomaterials** with different size, shape, and grafted fragments, including metal NP (Ag, Au, Fe), metal oxide NP (TiO_2, SnO_2).
- **Characterization** of the nanomaterials by physicochemical methods (diffuse-reflectance IR-Fourier spectroscopy, XPS, XAFS (EXAFS, XANES), TEM, XRD analysis, UV-visible spectroscopy, and plasmon resonance spectroscopy)
- **Toxicity tests** with aquatic organisms (daphnids, mussels).

METHODOLOGY-2

Modelling studies:

- **Statistical experimental design**
- **Accumulation and digestion of the available literature and own experimental data** on the toxicity of nanomaterials and their chemical composition, size, shape (morphology) and the availability and nature of the grafted groups.
- **Development of a user-friendly database** containing experimental physico-chemical and biological properties of nano-particles as well as integrating tools and computational methods to develop and publish in silico models of these properties.
- **Evaluation of predictability** of the toxicity of novel nanomaterials on the basis of newly developed algorithms linking physicochemical and modelled properties to the observed toxicity profiles of the NPs.

Expected impacts:

- The predictive models will help to prevent the ecological consequences of using nanomaterials as biocides, coating and cosmetic ingredients.
- Reduced need for empirical testing (reduction of costs, reduced need for animal testing).
- Nanomaterials that are safe by design will be predicted.

Helmholtz Zentrum and eADMET

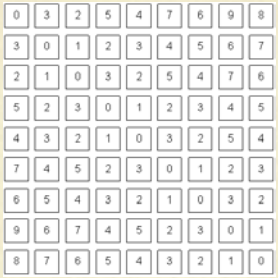
Helmholtz Zentrum München:
Ideas exploitation, research

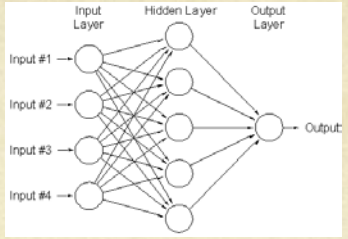
eADMET GmbH:
Development, commercial exploitation and industry/academy feedback

OCHEM – On-line CHEmical database and Modelling environment

- Database of molecules and their properties
- Advanced extendable modelling framework
- Hidden and private data and models
- Advanced modelling framework
- Public, open and community driven
- Scalable and extendable
- API to expose major functionality

How OCHEM parts interact?



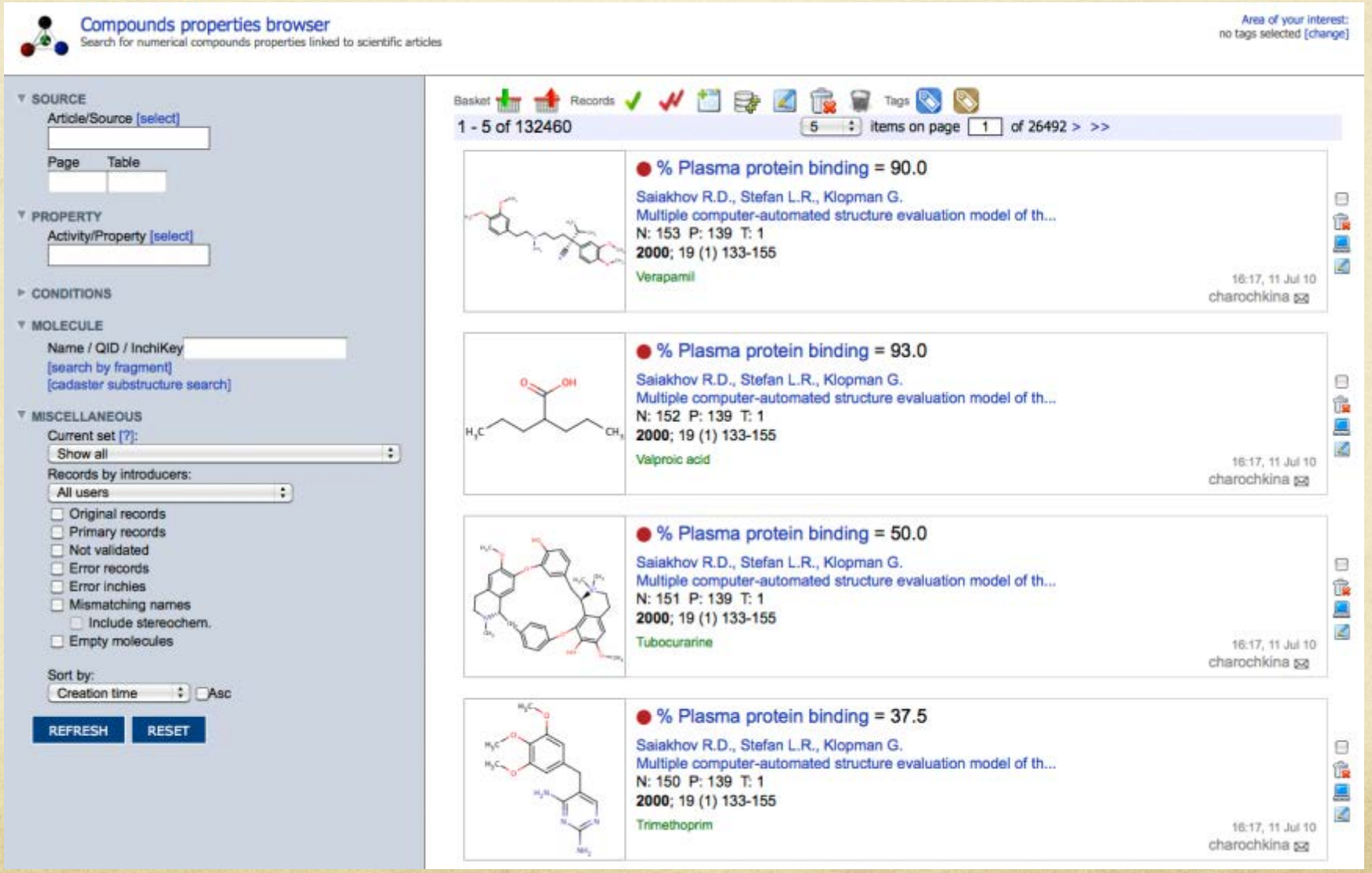


Database
Chemical structures (2D, 3D)
Experimental results, cached descriptors
Biological Pathways
Properties, their units, unit conversion rules, etc…

Descriptors
Chemical-structure-derived descriptor (in silico)
Biologically derived descriptor (in vitro)
Descriptors derived from Protein-ligand interaction (Autodock)
In research: Shape-derived descriptor (ex: for nanoparticles toxicity)

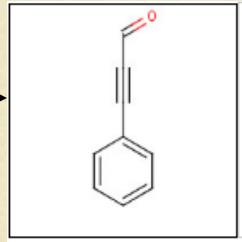
Mining algorithms
Linear and non-linear
Applicability domain estimation
Apply bootstrapping (bagging) and cross validation cycles
Model multiple properties simultaneously
Ex: ANN, KNN, SVM, J48

OCHEM – On-line Chemical Modeling Environment http://ochem.eu



Database schema
Simplified overview

Evidences



log(IGC50-1) = 2.02 -log (mmol/L) Temperature = 25.0

Zhu, H

Combinatorial QSAR modeling of chemical toxicants tested aga...

N: 445

Journal of chemical information and modeling **2008**; 48 (4) 766-84

2579-22-8 , phenylpropargyl aldehyde midnighter / itetko

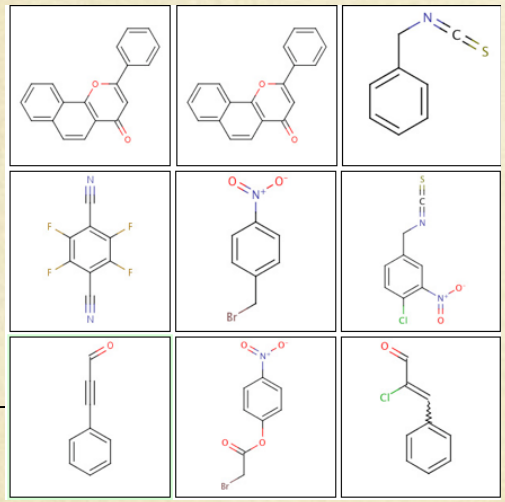
Properties

log(IGC50-1)	(concentration)	1093 records
LogPsuv	(dimensionless)	21 records
LogPsuv(ion)	(dimensionless)	21 records
LogPl	(dimensionless)	35 records

Conditions

species	(dimensionless)
Temperature	(temperature)
dose	(concentration)
Concentration	(concentration)

Molecules



Names

Users

Articles

Spink, DC;Spink, BC;Zhuo, X;Hussain, MM;Gierthy, JF;Ding, X;

NADPH- and hydroperoxide-supported 17beta-estradiol hydroxylation catalyzed by a variant form (432L, 453S) of human cytochrome P450 1B1.

The Journal of steroid biochemistry and molecular biology **2000**; 74 (1-2) 11-8

PubMed - ArticleID: Q1352

Zhang, L;Zhu, H;Oprea, TI;Golbraikh, A;Tropsha, A;

QSAR modeling of the blood-brain barrier permeability for diverse organic compounds.

Pharmaceutical research **2008**; 25 (8) 1902-14

DOI - PubMed - ArticleID: Q1577

Zhu, H;Tropsha, A;Fourches, D;Varnek, A;Papa, E;Gramatica, P;Oberg, T;Dao, P;Cherkasov, A;Tetko, IV;

Combinatorial QSAR modeling of chemical toxicants tested against Tetrahymena pyriformis.

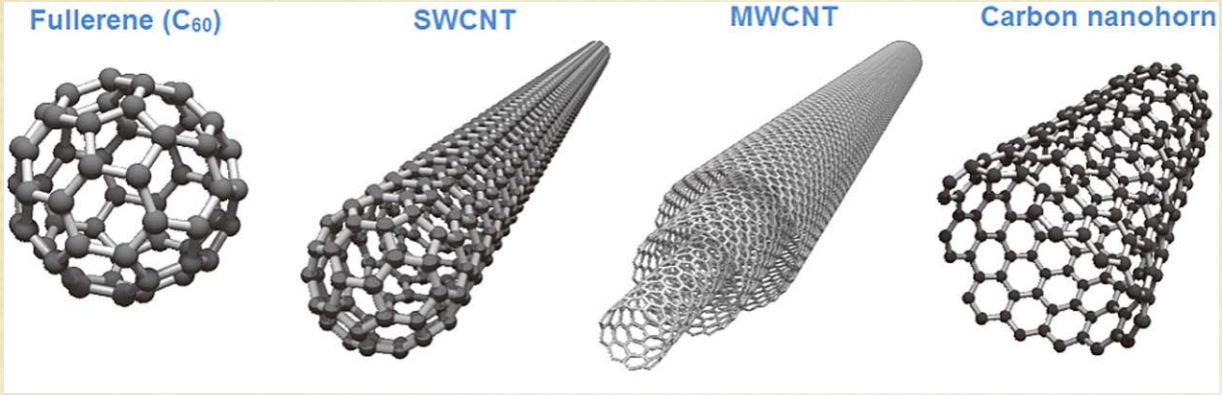
Journal of chemical information and modeling **2008**; 48 (4) 766-84

DOI - PubMed - PrePrint - ArticleID: Q1994

Units

log(mmol/L)	(concentration)
-log(mg/l)	(concentration)
nM	(concentration)
-log (mmol/L)	(concentration)

Nano-materials characterization



There is a need for a unique representation (language) to characterize nanomaterials

Small molecules:

- SDF, SMILES or other formats are used for traditional chemistry
- This representation is used to derive descriptors, i.e. to obtain representation of chemical structures suitable for machine learning methods

Representation of nanomaterials is a critically important for Nano-QSAR/QSPR.

“Nano” activities

http://www.eco-itn.eu

Overview of collected data during ECO fellowship of Miss N. Golovina

Table 1. Overview of the collected data.

NanoToxicity LC50 aquatic	89 records	13 compounds
NanoToxicity MIC	101 records	7 compounds
NanoToxicity immobilization	25 records	1 compounds
NanoToxicity mortality	75 records	5 compounds
NanoToxicity log(1/EC50)	17 records	17 compounds
NanoToxicity LC20 aquatic	15 records	5 compounds
NanoToxicity LD50	11 records	8 compounds
NanoToxicity EC50	21 records	8 compounds
Nanotoxicity survival	14 records	10 compounds
Nanotoxicity cell viability	32 records	1 compounds
NP aggregation state	3 records	1 compounds
Nanotoxicity [*OH] generation	48 records	2 compounds
NanoToxicity NOEC	20 records	5 compounds
NanoToxicity EC20	6 records	4 compounds

“Nano” data in OCHEM database

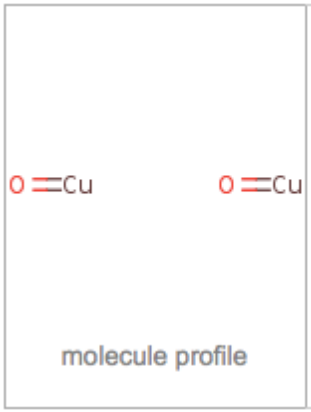
create new property | create new group

Type part of name to filter: nano search | Properties from other users: Only approved properties | Calculate counts

1 - 14 of 14

NanoToxicity NOEC	(Concentration / mg/L)	Show records	NanoToxicity NOEC value is designed as the highest tested c ...	natalia (moderator) / itetko
Biodistribution of nanoparticles	(Concentration / mg/kg)	Show records	Biodistribution of nanoparticles is distribution of nano pa ...	natalia / unmoderated
Nanotoxicity cell viability	(Dimensionless / %)	Show records	Viability of cells, the status of a cell to survive, grow, ...	natalia / unmoderated
NanoToxicity EC20	(Concentration / mg/L)	Show records	The term half maximal effective concentration (EC20) refers ...	natalia (moderator) / itetko
NanoToxicity EC50	(Concentration / mg/L)	Show records	The term half maximal effective concentration (EC50) refers ...	natalia / unmoderated
NanoToxicity immobilization	(Dimensionless / %)	Show records	NanoToxicity immobilization is inability to move. Animals t ...	natalia / unmoderated
NanoToxicity LC20 aquatic	(Concentration / mM)	Show records	Property NanoToxicity LC20 aquatic is determined as the let ...	natalia / unmoderated
NanoToxicity LC50 aquatic	(Concentration / mg/L)	Show records	Property NanoToxicity LC50 aquatic is acute endpoint for aq ...	natalia / unmoderated
NanoToxicity LD50	(Concentration / mg/L)	Show records	The median lethal dose, LD50 (abbreviation for Lethal Dose, ...	natalia / unmoderated
NanoToxicity log(1/EC50)	(Concentration / -log(mol/L))	Show records	The term half maximal effective concentration (EC50) refers ...	natalia / novserj / unmoderated
NanoToxicity MIC	(Concentration / ug/ml)	Show records	The minimum inhibitory concentration is the lowest concentr ...	natalia / unmoderated
NanoToxicity mortality	(Dimensionless / %)	Show records	Mortality rate is a measure of the number of deaths (in gen ...	natalia / unmoderated
Nanotoxicity survival	(Dimensionless / %)	Show records	Survival analysis is a branch of statistics which deals wit ...	natalia / unmoderated
Nanotoxicity [*OH] generation	(Concentration / mol/L)	Show records	Nanotoxicity [*OH] generation is the concentration of the r ...	natalia (moderator) / itetko

“Nano” records in OCHEM



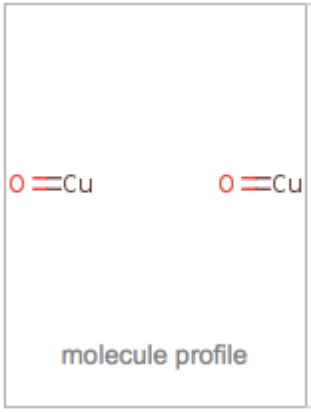
NanoToxicity EC50 = 79.0 (in mg/L)

Heinlaan, M et al
Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria...
N: AUTO_42 P: 4 T: 1
Chemosphere **2008**; 71 (7) 1308-16

MoleculeID: *M2652894*

Species = Vibrio fischeri
Test duration = 30min
Material Nanoparticles of Elements = Copper(II) oxide
APS= 30.0 nano meter
Shape of nano particles = N/A

RecordID: *R2707701*
16:01, 3 Apr 12 / 14:27, 9 Apr 12
natalia / itetko



NanoToxicity EC50 = 3811.0 (in mg/L)

Heinlaan, M et al
Toxicity of nanosized and bulk ZnO, CuO and TiO2 to bacteria...
N: AUTO_41 P: 4 T: 1
Chemosphere **2008**; 71 (7) 1308-16

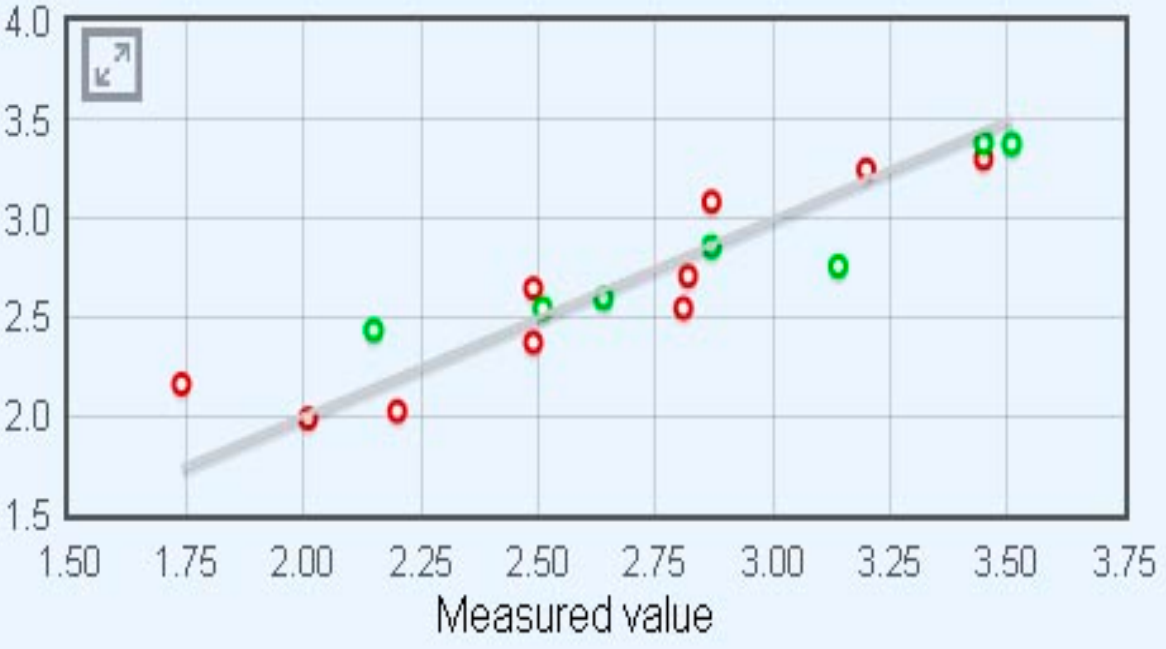
MoleculeID: *M2652894*

Species = Vibrio fischeri
Test duration = 30min
Material Nanoparticles of Elements = Bulk
APS> 100.0 nano meter
Shape of nano particles = N/A

RecordID: *R2707700*
16:01, 3 Apr 12 / 14:27, 9 Apr 12
natalia / itetko

Sample QSAR models for toxicity of nanomaterials

Data Set	#	R2	q2	RMSE	MAE
Training set: Puzin training	10 records	0.84	0.84	0.20	0.17
Test set: Puzin validation [x]	7 records	0.87	0.83	0.19	0.14



Data are from Puzyn et al, Nat Nanotechnol, 2011

Data used for model

molecule profile

● NanoToxicity log(1/EC50)
= 2.87 ± 0.13 (in -log(mol/L))

Material Nanoparticles of Elements = Lanthanum oxide
Cation formation enthalpy = 1017.22 kcal/mol

Puzyn, T et al
Using nano-QSAR to predict the cytotoxicity of metal oxide n...
N: AUTO_18 P: 2 T: 1 L: 17
Nat Nanotechnol **2011**; 6 (3) 175-8

MoleculeID: *M2652906*

RecordID: *R2655613*
17:51, 20 Feb 12
natalia

molecule profile

● NanoToxicity log(1/EC50)
= 2.51 ± 0.1 (in -log(mol/L))

Material Nanoparticles of Elements = Chromium(III) oxide
Cation formation enthalpy = 1268.7 kcal/mol

Puzyn, T et al
Using nano-QSAR to predict the cytotoxicity of metal oxide n...
N: AUTO_17 P: 2 T: 1 L: 16
Nat Nanotechnol **2011**; 6 (3) 175-8

MoleculeID: *M2652905*

RecordID: *R2655612*
17:51, 20 Feb 12
natalia

Even more “Nano” models

NanoToxicity MIC_ASNN_[], 156852	predicts NanoToxicity MIC using MIC_silver (83)	ANN	2012-12-02
NanoToxicity LC50 aquatic_ASNN_[], 156840	predicts NanoToxicity LC50 aquatic using 21 (35)	ANN	2013-03-12
NanoToxicity MIC_ASNN_[], 145066	predicts NanoToxicity MIC using MIC_silver (83)	ANN	2012-09-30
NanoToxicity LC50 aquatic_ASNN_[], 144159	predicts NanoToxicity LC50 aquatic using silver LC50 57 (19)	ANN	2012-09-19
NanoToxicity LC50 aquatic_ASNN_[], 144150	predicts NanoToxicity LC50 aquatic using silver LC50 57 (19)	ANN	2012-09-19
NanoToxicity immobilization, 39991	predicts NanoToxicity immobilization using Immobilisation_25 (25)	KNN	2012-05-02
NanoToxicity immobilization, 39989	predicts NanoToxicity immobilization using Immobilisation_25 (25)	FSMLR	2012-05-02
NanoToxicity immobilization, 39988	predicts NanoToxicity immobilization using Immobilisation_25 (25)	MLRA	2012-05-02
NanoToxicity immobilization, 39987	predicts NanoToxicity immobilization using Immobil_72h (16)	KNN	2012-05-02
NanoToxicity immobilization, 39986	predicts NanoToxicity immobilization using Immobil_72h (16)	FSMLR	2012-05-02
NanoToxicity immobilization, 39985	predicts NanoToxicity immobilization using Immobil_72h (16)	MLRA	2012-05-02

What we need to model nanotoxicity in OCHEM?

- Proper traceable definition of each nanomaterial (like SMILES string, IUPAC names)
  - Classification schema is required
  - Uncertainties in nanomaterials should be characterized
- Nano QSARs could be developed for well characterized and homogeneous classes of materials
  - Model for each class can be developed
- Alternatively toxicity could be assessed using experimental assays (i.e., similar to ToxCast project) to get assay-based representation of compounds
  - Proper selection of assays and understanding of DMKP (Drug Metabolism and Pharmacokinetics) is required
- **Minimum required information** to report nanotoxicity data can be defined

Prof. Peijnenburg Prof. Kustov Dr. L. Metelitsa