A battery of *in silico* NAMs to reliably reduce, refine and replace animal testing

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What are *in silico* NAMs?

New Approach Methodologies (NAMs) applying computational toxicology approaches to reliably predict the toxicity potential of chemicals.

Depending on the endpoint in question, they can be used either on their own or in combination with in vitro tests for both, R&D screening and regulatory submissions, provided the reliability of predicted results are justified adequately. using appropriate documentation.

They save significant time, cost and resources compared to traditional experimental methods however, their reliable application is often limited to mono-constituents.

How are they categorised?

Structural alerts (SARs)

Structure Activity Relationships (SARs) trigger alerts based on the presence of specific functional groups known to cause toxicity for the endpoint in question.

Commonly applied in the intial stage of R&D screening to phase out potentially toxic chemicals.

QSARs

Stands for Quantitative Structure Activity Relationships. They are trained on a large pool of experimental data (training set) often using statistically-sound algorithms to reliably predict the toxicity of new “structurally similar” chemicals (test set).

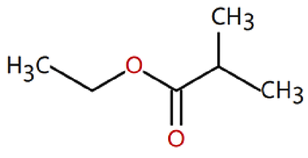
Particularly interesting for quantitative predictions (e.g. LD50 for Acute Oral or EC3 for Skin Sensitisation.

Read-across

Assigns prediction for a new chemical based on the experimental results for a category of structurally-similar chemicals (Analogues) with similar biological activities.

Popular choice for qualitative predictions (e.g. Skin irritant or non-irritant, Skin sensitiser or non-sensitiser)

Case study: Ethyl isobutyrate



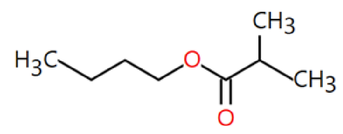
IUPAC Name: Ethyl 2-methylpropanoate
CAS: 97-62-1 | **EC Number:** 202-595-4
Canonical SMILES: CCOC(=O)C(C)C

Structural alerts (SAR) using OECD QSAR Toolbox v4.6:
Acute Oral Toxicity Profiler: **Not Categorised**
The target chemical does not match any of the criteria specified in this profiling scheme.

QSAR using CATMoS (via OPERA v2.9.1):
Model prediction: **GHS cat. 5 | LD50: 6,823 mg/kg bw**
Reliability: Inside Applicability Domain

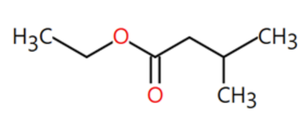
Read-across using OECD QSAR Toolbox v4.6:
Relevant analogues identified considering the structural, physicochemical, ADME and mechanistic similarities have exp. LD50 values >=5000 mg/kg bw (see references).

Analogue 1: Butyl isobutyrate
CAS: 97-87-0



Exp. data: 5,000 mg/kg bw
Rat Oral (Gavage)

Analogue 2: Ethyl isovalerate
CAS: 108-64-5



Exp. data: >5,000 mg/kg bw
Rat Oral (Unspecified)

Conclusion: Combining SAR/profiling, QSAR and Read-across results, the battery of *in silico* NAMs will not classify Ethyl isobutyrate for Acute Oral Toxicity. This is coherent with the experimental LD50 of >=5000 mg/kg bw provided in the ECHA dossier for this substance.

References:

- OPERA v2.9.1: https://ntp.niehs.nih.gov/whatwestudy/niceatm/comptox/ct-opera/opera
- OECD QSAR Toolbox v4.6: https://qsartoolbox.org/
- Ethyl isobutyrate (ECHA Dossier):https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/20388
- Butyl isobutyrate (exp.data for Acute Oral Toxicity): https://comptox.epa.gov/dashboard/chemical/hazard/DTXSID9073888
- Ethyl isovalerate (ECHA Dossier): https://echa.europa.eu/fr/registration-dossier/-/registered-dossier/21772