CASE STUDYS:
APPLYING ALTERNATIVE APPROACHES

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Case study 1: In-vitro methods

- Alternative methods have become OECD testing guidelines
  - In-vitro skin irritation -> OECD 439 since 2010
  - In-vitro eye irritation for non-irritants -> OECD 492 since July 2015

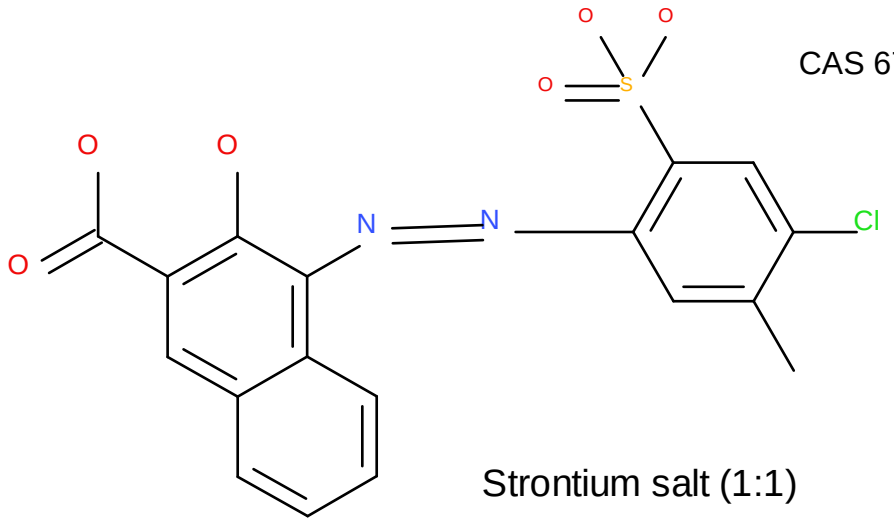
  - in-vitro/in-chemico skin sensitization assays since 2015 /2016

*Today these are **standard information requirements***

BASF studies were done under GLP and according to validated protocols

No challenge by ECHA so far

Case study 2: Structural similarity *with mechanistic considerations*



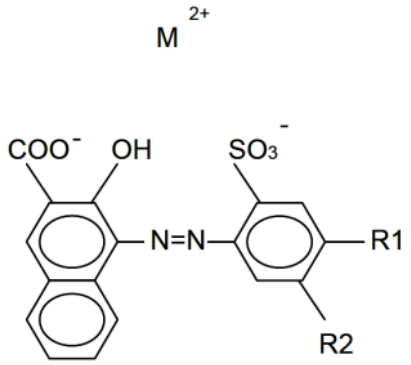
CAS 67828-72-2

Strontium salt (1:1)

<u>Literature/database search</u>
Substance itself
structural analogues
azo reduction products
soluble strontium salts

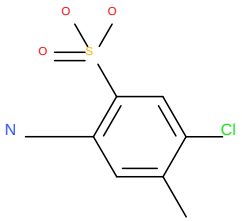
<u>OECD Toolbox / QSAR</u>
protein binding alerts
genotoxicity alerts
BfR inclusion/exclusion rules for irritation

Identification of structural analogues



	R1	R2	M
PR 57:1	CH_3	H	Ca
PR 57:Sr	CH_3	H	Sr
PR 48:1	CH_3	Cl	Ba
PR 48:2	CH_3	Cl	Ca
PR 48:3	CH_3	Cl	Sr
PR 48:4	CH_3	Cl	Mn
PR 52:Sr	Cl	CH_3	Sr
PR 52:1	Cl	CH_3	Ca
PR 52:2	Cl	CH_3	Mn

and the amine



Data matrix for Annex VII tox endpoints

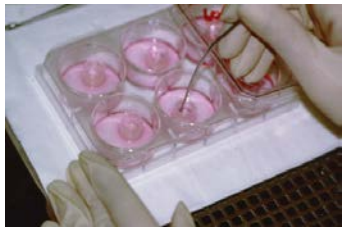
	PR 57:1	PR 57:Sr	PR 48:2	PR 48:1	PR 48:3	PR 48:4	PR 52:Sr	PR 52 amine
	Ca salt	Sr salt	Ca salt	Ba salt	Sr salt	Mn salt	Sr salt	
Melting point	decomp.	decomp.	decomp.	decomp.	decomp.	decomp.	decomp.	
Relative Density	1.7	1.8	1.7	2.0	1.9	1.7	1.78	
Water Solubility	1.25 mg/L	38 mg/L	0.26 mg/L	< 0.025 mg/L	0.11 mg/L	42 mg/L	< 15 µg/L	
Octanol solubility	5.5 mg/L	9.3 mg/L	0.047 mg/L	0.034 mg/L	0.065 mg/L	33 mg/L	0.2 mg/L	
skin irritation	not irritating	not irritating	not irritating	not irritating	(not irritating)	not irritating		
eye irritation	not irritating	not irritating	not irritating	not irritating	(not irritating)	not irritating		
skin sensitzation	not sensitizing		not sensitizing					
acute oral tox (LD50 in mg/kg bw)	> 5000	> 2200	> 5000	> 6400	> 2900	> 10000		> 2000
	No cases of mortality in any of the acute oral toxicity studies							
Subacute gavage study (OECD 407 or OECD 422) (NOEL in mg/kg bw)	< 100 (reversible kidney findings)	25 (reversible kidney findings)	40 (reversible kidney findings)					1000
Uptake/elimination	For oral application of high doses, both red staining of faeces and orange discolouration of urine are observed starting the first day of dosing and resolving within days after the last dose							
Bacterial mutagenicity	not mutagenic	not mutagenic	not mutagenic			not mutagenic		not mutagenic

WoE / read-across for acute oral toxicity

- Consistent hazard profile for structural analogues
  - covers β-naphthoic acid (BONA) and Strontium
  - No mortality observed at or above limit dose in acute oral studies
- Absence of hazard for sulfonated aromatic amine (acute and subacute oral study)
- Poor solubilities in water and octanol point to low uptake after ingestion, consistent with coloration of feces
- Azo bond reduction well described in the literature

WoE / read-across for irritation

- Consistent absence of hazard for structural analogues
- Differing substitutents do not influence pH or surface activity
- Consistent alert pattern (inclusion rules)
  - Only alert is «phenols», present in all analogues
- Physico-chemical parameters in support of «non irritating»
  - BfR Skin and Eye exclusion rules (not corrosive: octanol solubility of 0.2 mg/L and Tm> 200°C; not irritating: water solubility <5µg/L)



WoE / read-across for the Ames test

- Consistent hazard profile for structural analogues
  - covers BONA and Strontium
- Absence of hazard for differing sulfonated aromatic amine
- Azo bond reduction well described in the literature
- Ames tests done with Prival modification
- Comparable physico-chemical properties

Approach worked because…

- Physico-chemical data on all structural analogues available prior to decision making
- Letter of Access (LoA) / ownership for all data available (category)
- Data holders/registrants experienced in consortia work
- Robust study summaries for analogues already written

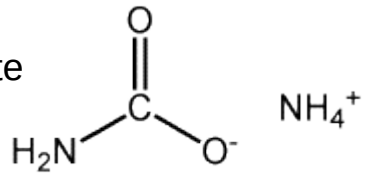
Also of note: Sweat equity costs spread over several endpoints

Other considerations

	standard information	***alternative strategy***
Number of Robust study summaries	one	one for each contributing study
Need for special justification	no	yes
Risk of challenge	no	yes
Suitability for other legislations (eg Water endangering class, sensitive application legislations, Korea-REACH, registrations outside EU, etc)	§ Accepted without discussion	Might not be accepted, or will require re-writing according to new templates/guidance, LoA for contributing studies if REACH-only.
Analytics and physico-chemical properties	needed for one substance	needed for any contributing substance

Case study 3:
Common dissociation products

Ammonium carbamate
CAS 1111-78-0



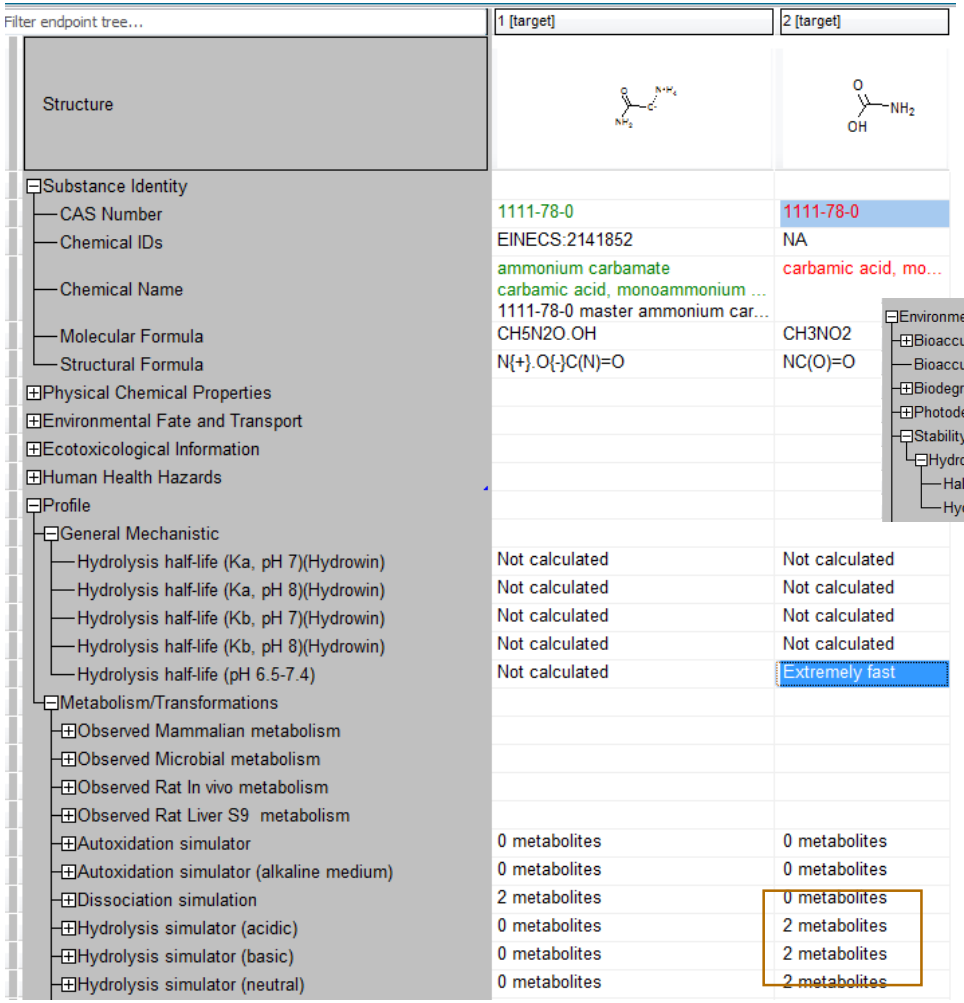
Behavior in aqueous solution:

Dissociation into NH_4^+, CO_3^{2-}, HCO_3^- (formation of several equilibria)

source substances:

- ammonium chloride
- ammonium hydrogen carbonate
- sodium hydrogen carbonate
- ammonium sulfate

Finding hints in the OECD toolbox



Filter endpoint tree…	1 [target]	2 [target]
Structure		
Substance Identity		
CAS Number	1111-78-0	1111-78-0
Chemical IDs	EINECS:2141852	NA
Chemical Name	ammonium carbamate carbamic acid, monoammonium … 1111-78-0 master ammonium car…	carbamic acid, mo…
Molecular Formula	CH5N2O.OH	CH3NO2
Structural Formula	N{+}.O{-}C(N)=O	NC(O)=O
Physical Chemical Properties		
Environmental Fate and Transport		
Ecotoxicological Information		
Human Health Hazards		
Profile		
General Mechanistic		
Hydrolysis half-life (Ka, pH 7)(Hydrowin)	Not calculated	Not calculated
Hydrolysis half-life (Ka, pH 8)(Hydrowin)	Not calculated	Not calculated
Hydrolysis half-life (Kb, pH 7)(Hydrowin)	Not calculated	Not calculated
Hydrolysis half-life (Kb, pH 8)(Hydrowin)	Not calculated	Not calculated
Hydrolysis half-life (pH 6.5-7.4)	Not calculated	Extremely fast
Metabolism/Transformations		
Observed Mammalian metabolism		
Observed Microbial metabolism		
Observed Rat In vivo metabolism		
Observed Rat Liver S9 metabolism		
Autoxidation simulator	0 metabolites	0 metabolites
Autoxidation simulator (alkaline medium)	0 metabolites	0 metabolites
Dissociation simulation	2 metabolites	0 metabolites
Hydrolysis simulator (acidic)	0 metabolites	2 metabolites
Hydrolysis simulator (basic)	0 metabolites	2 metabolites
Hydrolysis simulator (neutral)	0 metabolites	2 metabolites

Environmental Fate and Transport		
Bioaccumulation: Aquatic		
Bioaccumulation: Terrestrial		
Biodegradation	(1/1)	M: >80 %
Photodegradation		
Stability in Water		
Hydrolysis		
Half-life	(1/1)	M: <1.39E-5 Days
Hydrolysis Rate Constant		

… or talk to your production chemist

Human health endpoints

- Alternative assessment applied for
  - Acute inhalation toxicity
  - Clastogenicity
  - 90 day study (oral)
  - Teratogenicity(oral)
- Available studies on the substance itself:
  - Ames, HPRT
  - Acute oral and dermal toxicity (H302)
  - Skin and eye irritation (H318)
  - Skin sensitization
  - Physico-chemical properties (high water solubility, solid that decomposes prior to melting)

Bridging study requested for proof of concept

- $^{13}C\{^{1}H\}$-NMR in D_20 solutions of ammonium carbamate
  - 1% (pH 9-9,5) → complete dissociation within several minutes
    10% → formation of an equilibrium, no complete dissociation
  - 10%, acidified with DCl (stomach acid)→ complete dissociation immediately after acidification

Approach was easy because

- No relevant by-products/impurities
- Data on source substances published or available at BASF
- Expertise and experience available (regulatory and experimental)
- Reaction chemistry described in the literature
- OECD SIDS available for source substances

Faurholt C (1921). Über die Prozesse NH2COONH4 + H2O ↔ (NH4)2CO3 und CO2 + H2O ↔ H2CO3. Kopenhagen, Chemisches Laboratorium der köngl. tierärztlichen und landwirtschaftlichen Hochschule: p. 85-102 (in german).

Summary

Alternative approaches

- Offer an opportunity to avoid (animal) testing
- Can only be applied under certain conditions
- Require expertise in chemistry/toxicology/analytics
- Require extensive efforts in documentation, LoA and justification
- Are a moving target