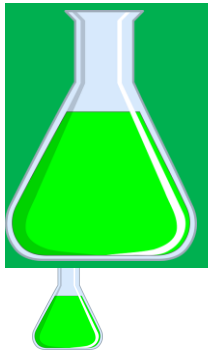
ASSESSING ALTERNATIVES IN VIEW OF UNCERTAINTY IN TOXICOLOGICAL DATA: NEW APPROACH METHODS (NAMS)

HANS PLUGGE

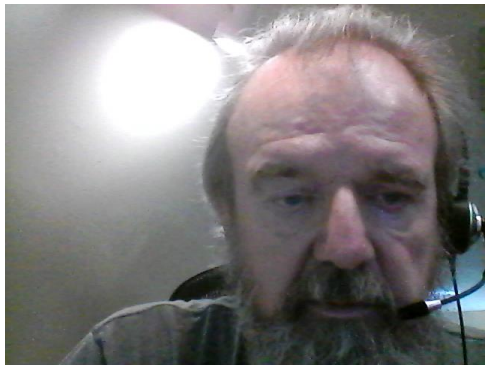


SAFER CHEMICAL ANALYTICS LLC

NOVEMBER 2021

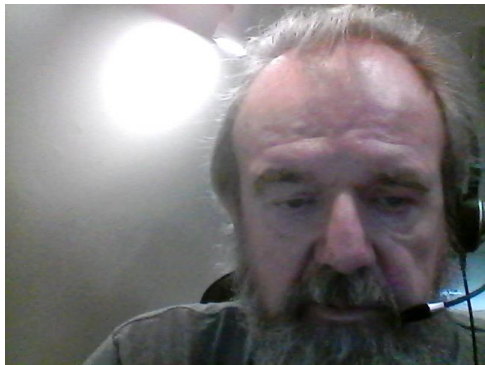
CONCLUSION

- New Approach Methods aka NAMs are presently used to screen “new” chemicals
- Due to varying restrictions on animal testing, NAMs may be the only regulatory methods available to assess the toxicity of alternatives
- Alternatives are thus mostly evaluated using NAMs
- In regulatory use however, NAMs have been penalized by high “uncertainty factors”
- Use of NAMs thus may result in rejection of alternatives at the regulatory level even though initial AA may have been favorable



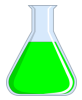
ALTERNATIVES ASSESSMENT (AA)

- AA of chemicals or processes
- Introduction of alternative, better and greener chemicals driven by increasing regulatory pressure and introduction of “new” toxicological data
- AA evaluates the (eco)toxicological parameters including environmental persistence, of available or *de novo* alternatives
- Feasibility of Alternatives
- Maintaining Functionality in Alternatives is important
- “NEW” chemicals require toxicological data mostly derived using NAMs (New Approach Methods)



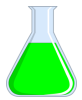
ACQUIRING NEW TOXICOLOGY DATA

- AA's by design incorporate lots of new chemicals/products
- New chemicals have little available data
- Traditional/old school toxicology data acquisition is expensive, if allowed
  - Animal testing is increasingly disallowed, except perhaps for ECHA
- NAMs were developed to address these concerns
  - Quicker, cheaper and more ethical
  - https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/alternative-test-methods-and-strategies-reduce
- Why not use NAMs for everything?
  - Regulatory acceptance versus validation
  - Lack of AOPs (Advanced Outcome Pathways) and assays



UNCERTAINTY

- Major concern: interpretation of NAMs re human/environmental risk
  - "addressed" via additional "uncertainty" factors
- Most NAMs are human/organism (*in vitro* cell) based tests
  - No need for animal to human extrapolation
- Traditional "gold standard" animal tests are much less certain than "expected"
- Variability/uncertainty for repeat animal tests is on at least an order of magnitude scale i.e., 3 implies somewhere between 1 and 10.
  - Pham et al.,2020 "Variability in in vivo studies: Defining the upper limit of performance for predictions of systemic effect levels," < https://doi.org/10.1016/j.comtox.2020.100126
- Conclusion: NAMs only have to be as good as traditional animal tests



SKIN SENSITIZATION AOP ADVERSE OUTCOME PATHWAY

Chemical properties	Molecular Initiating Event	Cellular Response	Tissue Response	Individual Population Response
• Electrophile or precursor • **QSARs**	• Covalent Skin Protein Interaction • Direct Peptide Reactivity Assay • **DPRA/QSARs**	• Dendritic Cells • **h-CLAT** • Keratinocytes • **KeratinoSens**	• T cell activation and proliferation • Local Lymph Node Assay • **LLNA**	• **Skin Sensitization**

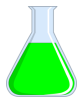
DEFINED APPROACH IS BETTER THAN HUMAN DATA

Model		CCR	Sensitivity	PPV	Specificity	NPV
Human	*in vivo*	0.82	0.94	0.86	0.70	0.86
LLNA	animal *in vivo*	0.59	0.65	0.71	0.54	0.47
DPRA	*in vitro*	0.67	0.84	0.75	0.50	0.64
KeratinoSense	*in vitro*	0.54	0.84	0.66	0.24	0.46
h-CLAT	*in vitro*	0.57	0.92	0.68	0.22	0.61
Bayesian Model	NAM	0.89	0.94	0.91	0.84	0.80

CCR: Correct Classification Rate PPV: Positive Predictive Value NPV: Negative Predictive Value

Adapted from Alvez et al 2018, https://www.researchgate.net/publication/323009353_A_Perspective_and_a_New_Integrated_Computational_Strategy_for_Skin_Sensitization_Assessment

Also see Golden et al 2020 , https://www.altex.org/index.php/altex/article/view/1492/2180



NAMS >= HUMAN DATA>>ANIMAL DATA

- Several of these approaches validate NAMs derived data as being equal or better than Human derived data
- NAMs are superior to Animal data
- ECHA very recently endorsed this approach

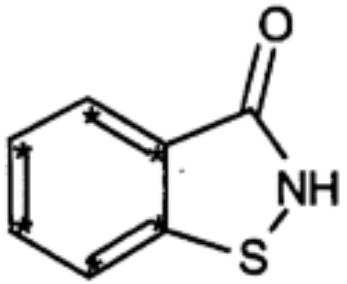
https://echa.europa.eu/documents/10162/21650280/oecd_test_guidelines_skin_sensitisation_en.pdf/40baa98d-fc4b-4bae-a26a-49f2b0d0cf63

- Skin sensitization NAMs are thus “solved”
- Similar approaches need to be developed/validated for other health and environmental endpoints – in progress

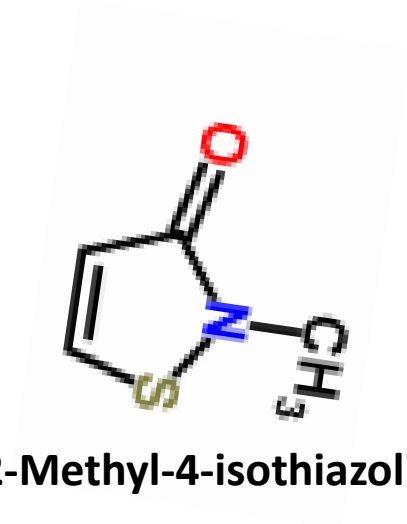
ISOTHIAZOLINONES CASE STUDY

- USEPA OPP (Office of Pesticide Programs) test case
  - https://www.regulations.gov/document/EPA-HQ-OPP-2015-0736-0008
- Isothiazolinones
  - a new class of biocide
- Skin sensitization data were derived from NAMs in a Defined Approach
  - Hirota M, et al. (2015) Evaluation of combinations of *in vitro* sensitization test descriptors for the artificial neural network-based risk assessment model of skin sensitization. Journal of Applied Toxicology 35:1333-1347
- Systemic toxicity data from traditional, animal test methods

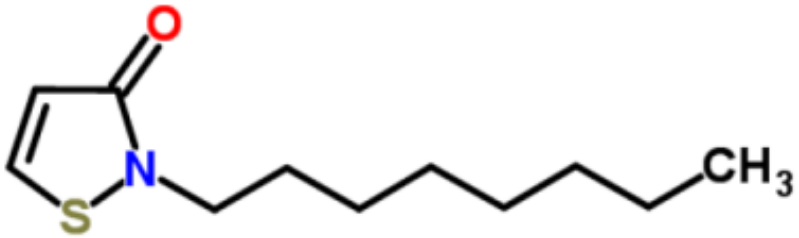
SELECTED ISOTHIAZOLINONE STRUCTURES



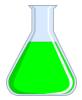
BIT 1,2 Benzisothiazolin-3-one

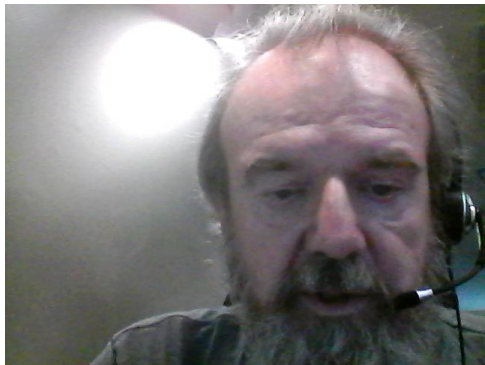


MIT 2-Methyl-4-isothiazolin-3-one



OIT 2-n-Octyl-4-isothiazolin-3-one





NAMS VS LLNA DATA (FROM USEPA)

Table 5. Quantitative EC3 Prediction for Isothiazolinones (Extracted from Table 7 of the NTP/NICEATM Report)

Chemical	Dow LLNA EC3 (%)	NICEATM LLNA EC3 (%)[a]	DA: ANN D hC[b] EC3 (%)[a]	DA: ANN D hC KS[c] EC3 (%)[a]
DCOIT	0.004	0.008 (0-0.053)	0.0566 (0.0555 – 0.0578)	0.023 (0.02 – 0.026)
CMIT/MIT	0.002	0.018 (0.0011-0.034)	0.121 (0.119 – 0.123)	0.492 (0.4 – 0.605)
OIT	0.2-0.25	0.361 (0.029-0.69)	0.0569 (0.0559 – 0.058)	0.015 (0.013 – 0.017)
MIT	0.863	1.154 (0-3.476)	1.775 (1.732 – 1.818)	0.826 (0.759 – 0.9)
BIT	1.54	10.57 (0-23.36)	0.934 (0.909 – 0.959)	0.341 (0.317 – 0.367)
BBIT	NA	NA	0.148 (0.146 – 0.151)	0.061 (0.055 - 0.068)

[a] Numbers in parentheses are the 95% confidence limits

[b] Model 1 from Hirota *et al.*, 2015: DPRA + h-CLAT

[c] Model 4 from Hirota *et al.*, 2015: DPRA + h-CLAT + KeratinoSens

Data from https://www.regulations.gov/document/EPA-HQ-OPP-2015-0736-0008

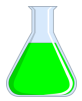
UNCERTAINTY FACTORS

BIT	**Induction:** Average *in vitro* EC3 = 0.34% (85 µg/cm^2) 95% Confidence Interval = 0.32 to 0.37%	UF = 100x (UF_A = 10X, UF_H = 10X)	Based on Model 4 from Hirota *et al.* 2015: DPRA + h-CLAT + KeratinoSens

- **Uncertainty factor of 10 x 10 for extrapolation of NAMs for induction of skin sensitization (originally was 100 x 100)**
  - The use of induction threshold values for the other members of the isothiazolinone class utilizes an uncertainty factor of 100. This factor includes the inter-species extrapolation factor of 10 (sic:***since the data are based on animal studies***), and an intra-species factor of 10.
- **Vs 10-100 for animal acute/chronic tox data**
  - The use of intra species factor of 3!

NAMS IN REGULATORY USE AS SHOWN HERE

- Skin Sensitization has the most validated NAMs (>10)
- Concordance: NAMs >= humans
- On a regulatory level, NAMs data would have to indicate 10 times less toxicity as compared to animal/human tests to make for a viable alternative
- Better acceptance of NAMs validation would go far to diminish this penalty
- “uncertainty” factors are not based on data analytics
- ECHA guidance is similar



CONCLUSION

- NAMs are presently used to screen “new” chemicals
- Due to varying restrictions on animal testing, NAMs may be the only methods available to assess the toxicity of alternatives
- Alternatives are thus mostly assessed using NAMs
- In regulatory use NAMs have been penalized by high “uncertainty factors”
- Assessment of Alternatives thus may result in rejection of alternatives at the regulatory level even though initial AA may have been favorable
- Assessment (“uncertainty”) factors need to be based on (validation) analytics not ballpark estimates

THANK YOU!

- hans.plugge@saferchemicalanalyticsllc.com

(+1) 410-446-7986