Review of methods to generate genotoxicity information and their ability to provide insight into carcinogenicity risk assessments

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Introduction

In vitro assays and *in silico* models have a growing importance in toxicological risk assessment as regulations shift to reduce the requirement for testing on animals. Many of these non-animal methods are hazard classification systems which are not currently accepted in isolation for carcinogenic risk assessment; large uncertainty is introduced without ability to consider exposure, dose and mode of action of a chemical. Nevertheless, the information provided by these methods may be used as weight of evidence in a carcinogenic risk assessment. In this vein, we investigated whether this information can correlate to carcinogenic potency, and how it may be combined with expert knowledge to inform on a carcinogenic mode of action.

Dataset curation

882 chemicals containing International Agency for Research on Cancer (IARC) classifications were extracted from Vitic Nexus. Chemicals expected to be carcinogens (Group 1, 2A and 2B) or not classifiable (Group 3) were included, but not non-carcinogens (Group 4). Further curation identified those chemicals with *in vitro* mutagenicity (Ames) or chromosome damage (chromosome aberration, micronucleus) assay data (288 compounds) or TD50 values from the Lhasa Carcinogenicity Database (LCDB) (429). The final dataset contained 173 chemicals with both *in vitro* genotoxicity data and a TD50 value.

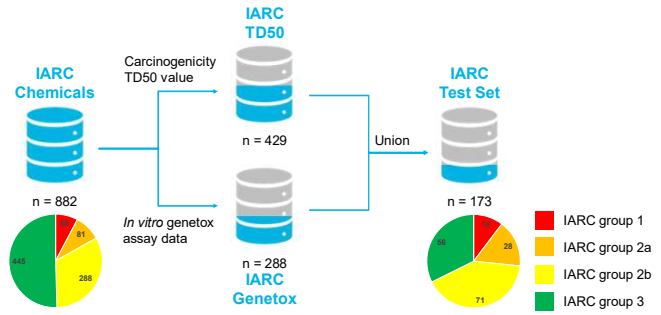

Figure 1. Curation of IARC dataset with proportion of chemicals in datasets per IARC group.

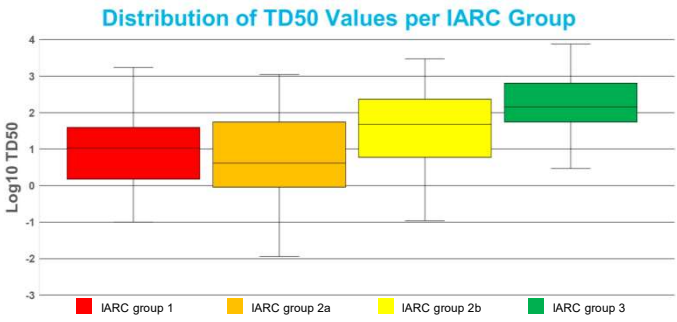

Figure 2. Box and whisker plot showing distribution of TD50 values per IARC Group for 173 chemicals in the IARC Test Set dataset. Outliers have been removed. All values entered for compounds tested multiple times.

- IARC Group 1 and 2a carcinogens are not distinguishable based on TD50 values.
- IARC Group 2b carcinogens overlap in potency with chemicals in Group 1, 2a and 3.
- IARC Group 3 chemicals are at least 1 order of magnitude less potent than Group 1 and 2a carcinogens.

Correlation of genetox data and carcinogenicity

- *In vitro* genetox assays are often concordant, most likely due to many chemicals in the test set being able to react directly with DNA.
- *In vitro* genetox assays are strongly predictive of IARC Group 1 and 2a carcinogens.
- Positive results for IARC Group 3 chemicals indicate genotoxic potential but do not assess *in vivo* relevance e.g., DNA-repair pathways.

Mutagenicity	Chromosome aberration +	Chromosome aberration -	Micronucleus +	Micronucleus -
+	106	14	50	9
-	22	25	11	7

Table 1. Correlation of in vitro genetox results for chemicals in IARC Test Set.

IARC group	Mutagenicity +	Mutagenicity -	Chromosome damage +	Chromosome damage -
1	15	3	17	1
2a	27	1	27	1
2b	48	23	54	17
3	35	21	44	12

Table 2. Correlation of IARC group with in vitro genetox assay data.

Chromosome damage = conservative call from chromosome aberration and micronucleus results.

Correlation of *in silico* and carcinogenicity

In silico predictions for carcinogenicity, mutagenicity (*in vitro*) and chromosome damage (*in vitro*) were made using Derek Nexus v.6.3.0 (Derek KB 2024 1.0) and the genotoxic mechanism was inferred from the accompanying alert description comments.

- Carcinogenicity is only predicted for chemicals in IARC Group 1, 2a or 2b.
- Mutagenicity (*in vitro*) predictions have high sensitivity carcinogenicity for IARC Group 1 and 2a chemicals (83% and 93% respectively).
- Chromosome damage (*in vitro*) predictions are less sensitive than mutagenicity for carcinogenicity (67% and 75% cf. 83% and 93%); limitations of these models is related to underlying data quality and quantity.
- Genetox predictions have low sensitivity for chemicals in IARC Group 2b and 3, and these are predicted less likely to bind to DNA, possibly suggesting non-genotoxic carcinogenic mechanisms are prevalent.

IARC Group	Chemicals	TD50 Range (Log10)	Number of Chemicals (%) Predicted Positive *In Silico* per Endpoint/Mechanism			
			Carcinogenicity	Mutagenicity (*in vitro*)	Chromosome Damage (*in vitro*)	DNA Binding
1	18	-4.63 – 2.82	18 (100%)	15 (83%)	12 (67%)	13 (72%)
2a	28	-1.96 – 3.04	25 (89%)	26 (93%)	21 (75%)	22 (79%)
2b	71	-0.73 – 3.42	51 (72%)	41 (58%)	40 (56%)	35 (49%)
3	56	-3.00 – 3.88	0 (0%)	30 (54%)	32 (57%)	23 (41%)

Table 3. Correlation of in silico predictions for carcinogenicity, mutagenicity (in vitro), chromosome damage (in vitro) and DNA binding. Predictions made using Derek Nexus v.6.3.0 (Derek KB 2024 1.0). DNA binding mechanism inferred from alert comments.

Using evidence to support carcinogenic risk assessments

Information from *in vitro* genetox assays and *in silico* predictions can be used to provide supporting evidence for carcinogenic risk assessments. Positive predictions for carcinogenicity from *in silico* systems in combination with negative results for genotoxicity can be rationalised with expert knowledge to assign non-genotoxic mechanisms of carcinogenicity.

- Dioxin is negative for genotoxicity *in vitro* and *in silico* but is indicated to bind to the aryl hydrocarbon receptor which induces carcinogenicity via the expression of several genes responsible for cell growth.
- Tetrafluoroethylene is negative for genotoxicity *in vitro* but predicted positive for mutagenicity *in silico*, additional information provided by Derek suggests tetrafluoroethylene may cause carcinogenicity by undergoing S-conjugation with glutathione resulting in intermediate species that react with DNA.

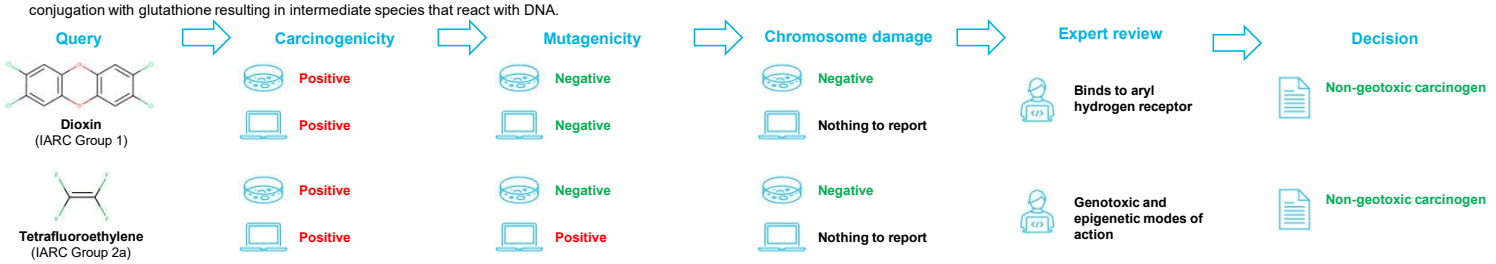

Figure 3. Workflow to show how in vitro assay data and in silico predictions can be used in combination with expert knowledge to provide evidence to support decision making for non-genotoxic chemicals in carcinogenic risk assessments.

Conclusions and future work

- There is limited correlation between IARC Groups and carcinogenic potency. *In vitro* genetox data and *in silico* predictions are predictive for IARC Group 1 and 2a carcinogens but less so for 2b and 3.
- All streams of information, in combination with expert knowledge of mechanism of action, can provide supporting evidence to make decisions on carcinogenic risk.
- Further analyses, including for non-carcinogens (IARC Group 4), will provide greater insight into how *in vitro* data and *in silico* predictions may be used to support carcinogenic risk assessment.

References

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References

Data set curation

❑Visit the Lhasa Limited website to find out more information about how Derek Nexus and Vitic can be used to support carcinogenicity assessments.

www.lhasalimited.org/solutions/carcinogenicity-assessment

❑ The Lhasa Carcinogenicity Database is a free to access source of long-term carcinogenicity studies founded on the now retired Carcinogenic Potency Database.

https://lcdb.lhasacloud.org/login

Correlation of genetox data and carcinogenicity

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Correlation of *in silico* and carcinogenicity

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Conclusions

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