Using historical negative control data to review Ames test results

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Introduction

The use of multiple criteria is recommended to establish whether a substance is mutagenic according to the Ames test e.g., a fold-increase in revertants or a concentration-response relationship, and expert review. Discussions at the 2017 International Workshop on Genotoxicity Testing (IWGT) meeting concluded that one of the most useful criteria is the consideration of historical negative control (HNC) data [1]. A follow-on publication (Levy et al., 2019) included an analysis of the Ames test to calculate HNC data for each strain and metabolic activation combination [2]. OECD Test Guideline No. 471 states that HNC data should be included in the test report for comparison with the concurrent control data as part of the Ames test acceptance criteria [3].

Datasets

Publicly available Ames test dose-response data from the Vitic database, (version 2019.1.0) [4], was used to create HNC datasets for Ames strains. The aim was to evaluate Ames test results in the Vitic database using these HNC datasets in combination with the other acceptance criteria. Using the Knime Analytics Platform® [5], data analytics was applied to create three datasets and perform the analysis, see Figure 1.

	No. of records	No. of records substances
Dataset 1: Untreated and vehicle (negative) controls selected to create HNC data (see figure 2).	17,244	1,037
Dataset 2: All untreated and vehicle (negative) controls from Ames test dose-response data.	97,638	4,257
Dataset 3: All Ames test positive results with dose-response data.	13,454	1,856

Figure 1: Three datasets were created from Ames test dose-response data in the Vitic database.

Figure 2 shows a workflow of how Dataset 1 was selected and filtered based on the experimental design consistent with the OECD Test Guideline No. 471 [3]. A control or confidence interval of 95% was used on the untreated and vehicle control values to calculate HNC data per strain, both with or without metabolic activation. This HNC data was used to analyse Datasets 2 and 3.

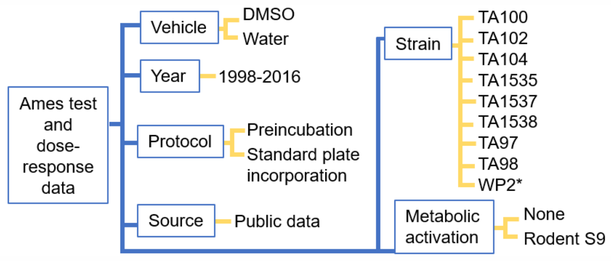

Figure 2: The selection criteria used to create Dataset 1 for Ames test HNC range calculations. WP2 is broken down into three groups* WP2 pKM101, WP2 *uvr*A and WP2 *uvr*A/pKM101.

Analysis of data

Table 1 shows the calculated HNC data for the concurrent strains and the number of records from Dataset 1 (a record being a single Ames test, in one strain, either with or without metabolic activation). These lower and upper limits, the HNC range, are plotted alongside HNC ranges from various literature sources, see Figure 3. The HNC ranges typically overlap the recommended historical ranges found in the published literature. However, a few of the calculated upper limits were above those indicated in the published literature, showing some variation in specific strains, e.g., the upper limits for *E. coli* WP2 *uvr*A +/-S9 were significantly larger than in published literature [6].

Strain	Mean		Historic negative control range				Number of records	
			Lower limit		Upper limit			
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
TA1535	14	13	7	7	29	24	1090	1580
TA1537	8	9	4	4	15	18	855	1089
TA98	20	26	12	15	37	42	1679	2349
TA100	115	125	58	58	170	190	1794	2520
TA97/TA97a	131	156	90	107	172	209	259	516
TA102	265	313	185	219	348	421	228	364
WP2 pKM101	121	152	55	47	172	205	29	29
WP2 *uvr*A	30	34	13	16	114	82	331	276
WP2 *uvr*A/pKM101	70	93	37	57	152	163	225	210

Table 1: Mean, upper and lower HNC values calculated from Dataset 1 for concurrent strains, +/- metabolic activation. The number of records used is also shown.

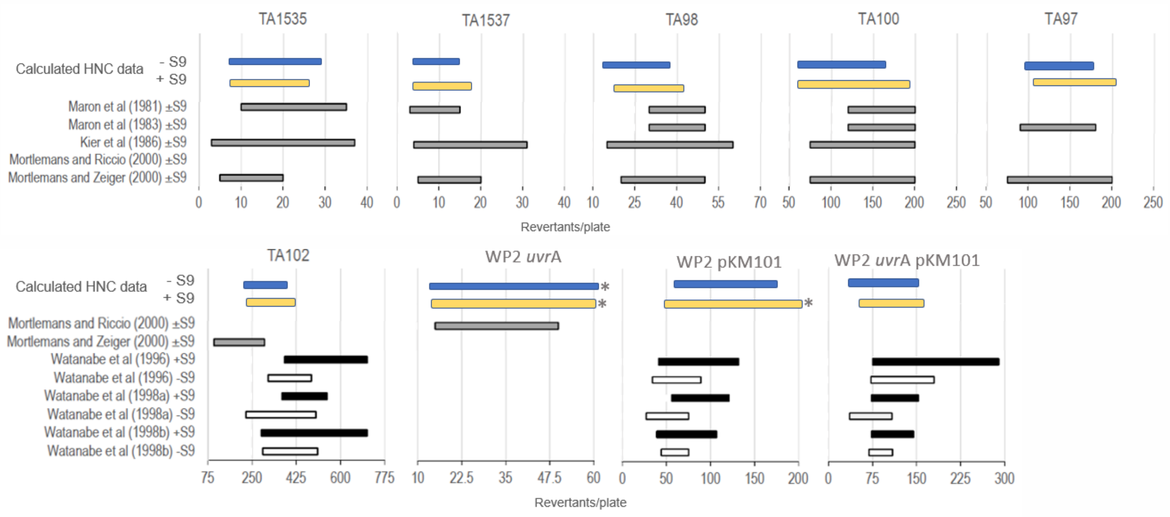

Figure 3: Comparison of recommended historical ranges found in published literature plotted against the HNC data for concurrent strains, with or without metabolic activation, calculated from Dataset 1. Where the upper limit extended past the right-hand side of the graph, it is indicated by an asterisk. [6], [7], [8], [9], [10], [11], [12], [13].

The HNC ranges were first used to highlight Ames test untreated and vehicle (negative) control values in Dataset 2, not within the HNC range as OECD Test Guideline No. 471 recommends [3]. It was observed that 10,903 records (11.2% of Dataset 2) had control values outside the HNC range, indicating possible issues with the dose-response data. The next step was to analyse the Ames test positive results (Dataset 3). 5.4% of the records had dose-response values below the upper limit of the respective HNC range, highlighting possible inconclusive positive responses. These inconclusive results required further investigation as there are multiple criteria that need to be considered when classifying an Ames test positive. 34 substances were prioritised for review in the Vitic database that did not have supporting positive Ames test results. To aid the review, the fold-increase in revertants, compared to the control was calculated from the records with dose-response data, as a 2- or 3-fold increase, dependent on the strain, can be used as an acceptance criteria for establishing a positive response.

Using the historical negative control data

The two examples in Figure 4 and 5 show images of data in the Vitic database and comments added to show the results from the HNC data analysis and fold-increase in revertants. For both entries, the Lhasa Ames summary call results are conflicted, and the genetic toxicity *in-vitro* table contains one weakly positive result that is causing the conflicting summary call [14].

Figure 4 shows data on lauric ethylolamide. The genetic toxicity *in-vitro* table dose-response results do not exceed the HNC range and the fold-increase in revertants does not exceed the 2-fold recommendation. The source's overall call for the substance is listed as negative. Using expert review and considering multiple criteria, we can conclude that this weakly positive result should not be considered in the Ames summary call.

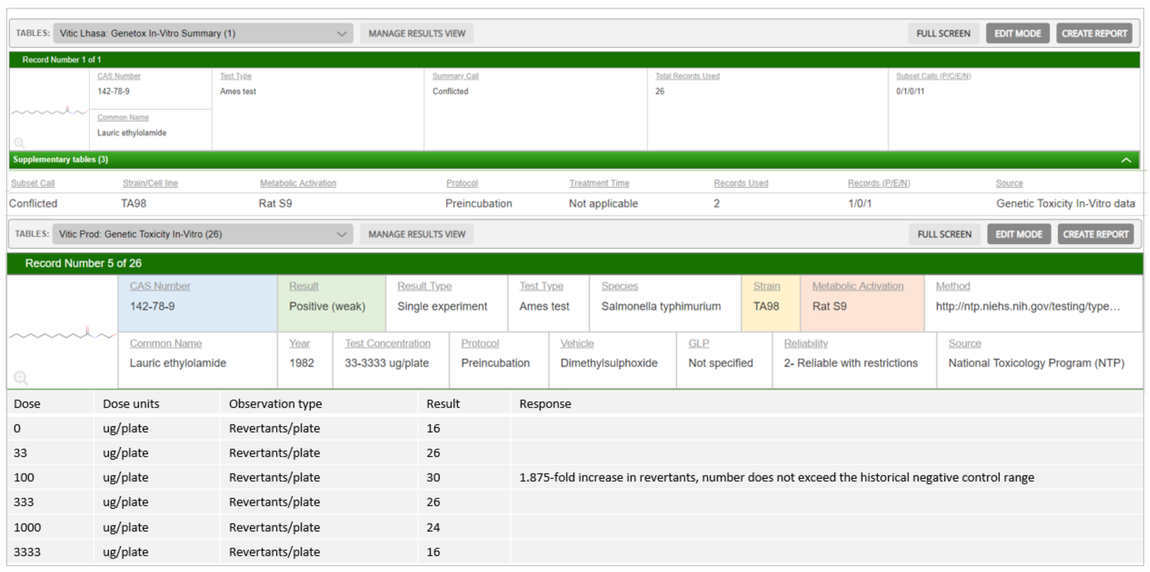

Figure 4: Data for lauric ethylolamide in Vitic database with HNC ranges and comments added sourced from National Toxicology Program [15].

The second example (Figure 5), shows data on 4-chloro-2-methylphenoxyacetic acid. The dose-response data shows the revertant count never exceeds the HNC range and the fold-increase in revertants is below the recommended 2-fold increase. Expert review could determine this result as inconclusive, and as all other Ames results for the substance are negative, the Ames summary call could be amended to negative.

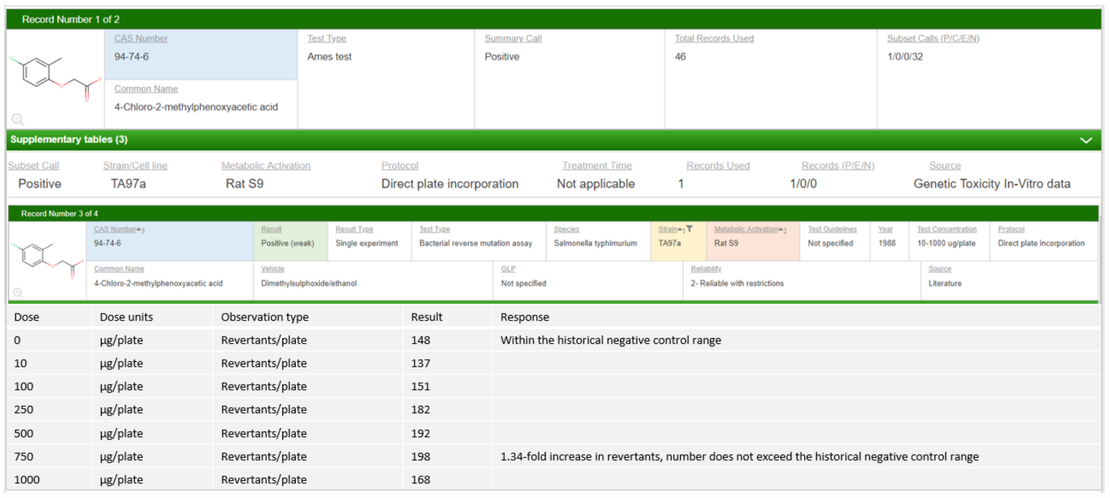

Figure 5: Data for 4-chloro-2-methylphenoxyacetic acid with HNC ranges and comments added.

Conclusions and future work

A large Ames test dataset can be used to create HNC data with a 95% confidence interval. The HNC ranges have been used to analyse the concurrent strains dose-response data and to identify Ames test data in the Vitic database that may require investigation. It flagged control data outside the HNC ranges and positive results that did not exceed the HNC range. Used alongside other acceptance criteria such as calculating the fold-increase in revertants, a review of specific records will aid users in their expert review and clearly show the multiple criteria used to review Ames test results, establishing whether a substance is mutagenic.

References

1] Martus et al (2020) https://doi.org/10.1016/j.mrgentox.2020.503134 [2] Levy et al (2019) https://doi.org/10.1016/j.mrgentox.2019.07.004 [3] https://www.oecd-ilibrary.org/environment/test-no-471-bacterial-reverse-mutation-test_9789264071247-en [4] Vitic (2019.1.0), Lhasa Limited https://www.lhasalimited.org/products/vitic.htm [5]KNIME.com AG (version 3.4.2). Available at: https://www.knime.org/. [6] Mortlemans and Riccio (2000) https://doi.org/10.1016/S0027-5107(00)00076-2 [7] Maron and Ames (1981) https://doi.org/10.1016/0165-1218(81)90025-2 [8] Maron and Ames (1983) https://doi.org/10.1016/0165-1161(83)90010-9 [9] Kier et al (1986) https://doi.org/10.1016/0165-1110(86)90002-3 [10] Mortlemans and Zeiger (2000) https://doi.org/10.1016/S0027-5107(00)00064-6 [11] Watanabe et al (1996) https://doi.org/10.1016/S0165-1161(96)90249-6 [12] Watanabe et al (1998a) https://doi.org/10.1016/S1383-5718(97)00155-1 [13] Watanabe et al (1998b) https://doi.org/10.1016/S0165-1161(96)90249-6[14] https://www.lhasalimited.org/publications/summation-of-toxicity-data-in-vitic/3918 [15] https://ntp.niehs.nih.gov/

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