How low can you go?
An analysis of lowest effective dose in the Ames test.
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Introduction
The food industry requires risk assessments to be performed on all substances migrating from Food Contact Articles (FCAs) and Food Contact Materials (FCMs). This includes Non-intentionally added substances (NIAS), which often form a significant part of the overall migrate [1]. The risk assessment of NIAS has proven difficult due partly to questions regarding the sensitivity of test methods and if the small amount of migrate produced is insufficient for toxicological testing. Risk assessments also strongly rely on available data and strategies are not in place for obtaining this data [2]. The results of in vitro tests could be used directly to highlight components that are problematic in terms of toxicological hazard [3], see figure 1.

Figure 1: Workflow showing FCMs and FCAs, extraction of migrate, hazard identification and incorporation of genotoxicity assays as part of the risk assessment.

The Ames test is a sensitive assay for mutagen detection and is one of the most common tests used for identifying potential mutagenic impurities [4]. Thus the question is whether the conventional Ames test is sufficiently sensitive to detect toxicity at very low doses?

Results
Following the analysis, the calculated LEDs ranged from approximately 0.001-20000 µg/plate. Substances with LEDs higher than 5000 µg/plate were not included in the dataset since this is the upper dose requirement in the OECD 471 guideline, resulting in a dataset of 1672 mutagenic substances. Summary statistical parameters such as mean, minimum and maximum were derived from the LED for each substance and the primary focus was on the minimum LED.

Figure 3 shows the minimum LEDs of substances in the dataset. 83% of results are clustered at concentrations between 0-500 µg/plate. A Box plot is also shown in figure 3 which presents the majority of the distribution of the minimum LEDs. The median minimum LED was 100 µg/plate and the interquartile range was 10-333 µg/plate.

Figure 3: Chart and Box plot demonstrating the distribution of minimum LEDs (µg/plate) of the substances.

Table 1 shows the 15 most potent substances as judged by their minimum LED. An asterisk indicates where the minimum LED was also the lowest dose tested i.e. it is possible that a lower dose may also produce a positive result, therefore a lower LED could be produced. However, in most cases the fold increase for the substances was close to 2-fold, suggesting that lower doses would produce insignificant responses.

Table 1: Substance, minimum LEDs (µg/plate) and the fold increase in revertants for most potent 15 results. Asterisk indicates if the dose tested was the lowest tested dose.

Conclusion
The Ames test can detect mutagens at low doses. A minimum LED as low as 0.001 µg/plate was observed and the vast majority of substances had minimum LEDs between 10-333 µg/plate. Most strains had a very similar sensitivity and could be used interchangeably. It was interesting that in this dataset several substances’ LEDs were also the lowest tested dose and further investigation could reveal even lower LEDs being produced. For NIAS a self-regulated threshold of 10 µg/kg, (10ppb) in food has been set by industry [5] and if exceeded, further risk assessment need to be performed. The Ames test has detected mutagens at ppb dosage. Thus its feasible that the FCM industry could incorporate the Ames test and Ames test data to detect and/or confirm potential mutagenicity safety hazards.

Method
To investigate this question, publicly available Ames test and dose response data for more than 4300 substances was collated from the Vitic® database [5]. Using Knime Analytics Platform® [6], data analytics was applied to determine the lowest dose at which a mutagenic response could be detected. Figure 2 shows a workflow of how the dataset was selected and filtered based on the experimental design consistent with the OECD 471 guideline [7]. The calculated lowest effective dose (LED) was defined as the dose that produced at least a 2-fold increase in revertant count compared to control.

Figure 2: Workflow showing the selection and filtering of the dataset from Vitic to calculate the LED. (*) TA97 and TA97a, WP2 uvrA and WP2 uvrApKm101 strains were grouped together.

Table: Substance, minimum LEDs (µg/plate) and the fold increase in revertants for most potent 15 results. Asterisk indicates if the dose tested was the lowest tested dose.

The dataset was also analysed per strain, to establish if any were more sensitive than others. Figure 4a shows that the “GC” strains have similar sensitivity (minimum LED median ~100 µg/plate, upper quartiles 333 µg/plate). However, figure 4b showed the “AT” strains to have a larger distribution of minimum LEDs (upper quartiles >>1000 µg/plate) and the E.coli WP2 uvrA strain was less sensitive (median minimum LED 375 µg/plate) than TA102.

Figure 4: Box plot with the outliers removed showing the distribution of minimum LEDs (µg/plate) by strain.

References