A new ICH M7 compliant expert alert system to predict the mutagenic potential of impurities

March 2014
A new ICH M7 compliant expert alert system to predict the mutagenic potential of impurities

Abstract
The current International Conference on Harmonisation (ICH) M7 guideline on drug impurities states that two distinct in silico methodologies can be used to qualify certain drug impurities as not mutagenic. This paper outlines the development and use of a new expert rule-based system to predict the results of a bacterial mutagenesis assay. In the development of this system, an initial library of mutagenicity structural alerts was identified from the literature. This process included consolidating the same or similar alerts cited in multiple publications. Information on plausible mechanisms was collected alongside the structural definitions. Factors that deactivate the alerts were also identified from the literature and through an analysis of the corresponding data using the Leadscope data mining software. Over 200 distinct alerts were identified and these alerts were further validated against a reference database of over 7,000 chemicals with known bacterial mutagenesis results. Only validated alerts with a sufficiently strong association with positive expert-reviewed calls from Salmonella and E. coli strains were included. A prediction of the bacterial mutagenesis assay can be made using these validated alerts; however, this is only possible when the compound is within the applicability domain of the alert system. In addition, a confidence score based upon information collected for each alert is provided alongside the positive or negative call. This paper outlines the expert alerts system and presents validation results, both as a standalone system and in combination with statistical-based approaches and available experimental data.

Introduction
Impurities are generated as part of the pharmaceutical manufacturing process. They result from using different reagents, catalysts and solvents in the synthesis of the drug substance and are also attributable to subsequent degradation. The International Committee on Harmonisation (ICH) has recently issued a draft guidance (currently at Step 2) that covers the qualification of mutagenic impurities [1]. The purpose of the guidance is to ensure that any impurities present in the drug substance pose a low-level of risk of causing cancer. As such, the guideline’s focus is on identifying DNA reactive substances, usually detected using the bacterial reverse mutation assay (Ames) [2]. In the absence of carcinogenicity or Ames data for the actual or potential impurities, a computational structure-activity analysis can be performed to help understand whether a substance can be classified as not mutagenic. Where this negative classification is not possible, further in vitro or in vivo tests are required to support a negative classification or the impurity should be controlled below the limits established in the guideline.

To perform the computational structure-activity analysis, the guideline states that two complementary in silico methodologies should be used in the assessment. One should be expert rule-based and the second should utilize a statistical-based methodology. The methods used in the assessment should be
compliant with the OECD validation principles [3] and should be reviewed using expert analysis, especially in cases where the results are conflicting. If the results from the two methodologies indicate no predicted mutagenic potential, the impurity can be classified as having no mutagenic concern.

An expert rule-based system is one of the methodologies required to support an ICH M7 compliant computational analysis. In this type of system, knowledge concerning different structural features that are associated with mutagenicity is encoded as rules in the system. If these structure-activity rules map onto the compound being evaluated, then it may indicate the compound is mutagenic. A number of current systems make use of this rule-based methodology including ToxTree[4], HazardExpert [5], DEREK [6,7], and Oncologic [8]. ToxTree is a rule-based system that incorporates a module for mutagenicity which includes the Benigni-Bossa rule-base. HazardExpert contains a mutagenicity module which makes use of a series of toxicophores from the literature. DEREK is an alerts-based system that incorporates mutagenicity rules and generates several levels of prediction including “Certain”, “Probably”, “Plausible”, “Equivocal”, “Doubted”, “Improbable”, “Impossible”, “Open”, “Contradicted”, and “Nothing to report”. At this time, there is no domain of applicability analysis and hence no negative predictions. Oncologic is an expert system that makes predictions based on a series of user decisions making it difficult to predict batches of chemicals.

The approaches discussed were not developed to specifically address the criteria of the ICH M7 guideline. One of the main disadvantages of all current approaches is their lack of an applicability domain assessment (one of the OECD validation principles). This is a particularly important part of a M7 submission, since the lack of an alert should never be used to state that a compound has no mutagenic concern. This would imply that all possible structural alerts have been identified and that chemistry is static. Aryl boronic acid is an example where a new structural alert has been recently identified [9].

In addition, these approaches are not generally quantitatively assessed using a large database of Ames results. This is important since the guideline explicitly states the necessity for the alerts system to be predictive of the bacterial mutation assay. This also supports transparency in the prediction results by providing the data used to assess and justify the structural alerts.

Decisions compliant with ICH M7 guidance require a clear positive or negative call. While less precise predictions may be helpful when using these tools in early discovery for prioritization, they are ambiguous for regulatory needs. Prediction results such as “Probable” or “Plausible” need to be translated into a positive/negative call. This level of ambiguity permits inconsistent application of these tools.

Finally, the tools described do not address the complex relationships between regulatory acceptable statistical model results, alert results, available experimental data and the necessity for expert opinion support tools that are essential when supporting the ICH M7 guideline.
The following paper outlines a new expert rule-based system designed to address the needs of regulators and pharmaceutical scientists who must implement the ICH M7 guidance. The alert knowledge base is built using a quantitative assessment of publicly-cited alerts using a large database of Ames results. For each alert, deactivating factors are enumerated through an analysis of the available Ames data and the literature. The alerts are organized hierarchically, to precisely define the relationship between any alerts with any specific classes of concern. This rule base is annotated with plausible mechanisms collected from the literature. This linkage between the data and the biological interpretation of the alerts is critical to support a transparent assessment of the results. This paper outlines the methods used to build the alerts knowledge base and the methodology used to make predictions. Validation results are presented and a discussion of its use to support the ICH M7 guidance is provided.

Methods

Reference set
An extensive high quality genetic toxicity database containing the results of the bacterial mutagenesis assay along with chemical structures has been used to support the development of this rule-based expert system. This database, referred to as a reference set, supports the evaluation of the expert rules. The use of the term reference set is to contrast this database from a training set used in QSAR model development. This set will not be used to ‘build’ the alerts system, rather it will be used (1) to assess the alerts cited in the literature (in combination with an expert judgment), (2) to support applicability domain assessment and (3) to generate scores that are reflective of the confidence in the alert.

Two high quality data bases were used, each comprised of data from numerous sources: the training sets used to build QSAR models at the US FDA with Research Collaboration Agreement partners (RCA-QSAR) [10,11] and the Leadscope SAR 2014 database [12,13].

The RCA-QSAR database is comprised of 3,979 chemicals, with data on overall Salmonella strains as well as 1,198 chemicals with a composite TA102 / E. coli strain call. These datasets contain non-proprietary data harvested from FDA approval packages and the published literature.

The Leadscope SAR 2014 Genetox Database is an extensive collection of genetic toxicology studies (including bacterial mutagenesis, chromosome aberration, mammalian mutagenesis, and in vivo micronucleus) and contains 6,805 chemicals with graded bacterial mutagenesis calls. Information was collected from the electronic source or manually harvested to create the data set. Specifically, the following sources were used: (1) the US Food and Drug Administration (FDA) Centre for Food Additives and Applied Nutrition (CFSAN) Food Additive Resource Management system (FARM) and Priority based Assessment of Food Additives (PAFA) [14]; (2) the US FDA’s Center for Drug Evaluation and Research (CDER) Pharmacology Reviews based on the new drug approval (NDA) documents [15]; (3) the Chemical
Carcinogenicity Research Information System (CCRIS) [16]; (4) the National Toxicology Programs (NTP) genetic toxicology database [17]; (5) the Tokyo-Eiken database [18]; (6) and other publications.

The two databases were combined for the purposes of developing a comprehensive reference set for use in supporting the development and application of the expert rule-based system. The process of combining the database was performed in two steps. Firstly, the chemical structures were merged through using a chemical registration system to assign a unique identifier to each chemical and merging entries on the basis of this identifier. Next the graded endpoints for Salmonella and E. coli were combined from the different sources, resulting in a database of over 7,000 chemicals each with a positive/negative overall bacterial mutation call. The distribution of positive and negative data is summarized in Figure 1. The reference set also covers a diverse collection of compounds since they have been derived from many different sources, including pharmaceuticals, pesticides, industrial chemicals and food additives. This diversity is illustrated by clustering the reference set using the Leadscope software, using a cut-off distance of 0.5 [19-24]. A summary of 4 out of the 2,269 clusters is presented in Figure 2. There were 1,220 clusters with two or more examples, and 1,049 singletons (clusters with one example).
Alert compilation

A bacterial mutagenesis structural alert is based on a molecular substructure defining a reactive center, as illustrated in Figure 3. In this figure, an aziridine substructure is shown which had been cited in multiple publications as a structural alert for mutagenicity ("aromatic and aliphatic aziridinyl" [25]; "aziridine" [26]; "oxiranes and aziridines" [27]; "SA_7: epoxides and aziridines" [28,29]). For any mutagenicity structural alert, there should be a relationship between this reactive center identified within the molecule and its ability to either directly or indirectly (through one or more metabolic steps) interact with DNA. For example, in [29] aziridines have been described as “… extremely reactive alkylating agents that may react by ring-opening reactions … activity of these compounds depends on their ability to act as DNA cross-linking agents, via nucleophilic ring-opening of the aziridine moiety by N7 positions of purines.”

A number of publications have published summaries of proposed alerts [25-29,31-34]. The first step in the process of developing an expert alert-based system is to encode these published alerts as substructural definitions. This involves defining the one or more substructures that define the alerts. Many publications also include a description of the mechanistic basis for the proposed alerts and this rational is captured alongside the structural definitions.

Next, the alerts are consolidated wherever possible; however, this process is challenging since not all publications define the same alert in the same way. This is illustrated in Figure 4 where four papers cite the alert aziridine in different ways. The Ashby 1988 paper has substitutions on both carbons and any substitutions on the nitrogen is ambiguous [25], the Kazius 2005 paper places no restrictions on any attachments [26], the Bailey 2005 and Benigni 2008 & 2011 papers’ definitions include both epoxides/oxiranes in the same definition [27-29]; however, no restrictions on any of the atoms are presented in Bailey but in the Benigni papers the nitrogen should have one attachment to any atom. The process of consolidating the alerts must take into account the different definitions and ways the alert have been defined. It was performed on a case-by-case basis. The plausible mechanisms are also helpful in refining the alerts’ definitions.
Once these alerts have been encoded and consolidated, they are organized hierarchically, as illustrated in Figure 5. In this example, the alert 28: nitrosamine is a parent alert, with three more specific child alerts related to it. There are several reasons for doing this. Firstly, it helps in establishing a mechanistic explanation, particularly where any child alert is lacking or has limited mechanistic information, as it may be inherited from the parent alert. Secondly, when the expert alerts are used to make prediction, a score is calculated reflecting the precision of the alert. If more than one alert matches the target compounds being assessed, then the most precisely defined alert (i.e. the alert in that is closest to any terminal node in the hierarchy) is used to generate this score. Finally, providing a summary of the alert(s) that fire is useful; however, a list of many related alerts is not helpful. This hierarchy can be used to select the most precise and relevant alert to present.
In addition to the primary alert, it is also important to define any factors that would deactivate the alerts as a result of electronic or steric effects or by blocking an important metabolic step. For example, for the alert *primary aromatic amine*, an acidic group in the para position “…prevents proton abstraction from NH2 group of anilines … by the ferric peroxo intermediate of CYP1A2.” [30]. Unfortunately, there is limited information in the literature on precise factors that deactivate the alert, therefore, an exercise of data mining the reference set was undertaken to better understand these factors. This process used the informatics tools available in the Leadscope software to identify and quantitatively assess deactivating factors [19-24]. This process used the 27,000 pre-defined structural features in Leadscope and generated new chemical scaffolds associated with negative bacterial mutagenicity. Any deactivating fragments identified were quantitatively evaluated using the reference set. Figure 6 illustrates three example deactivating fragments for the alert *aromatic nitro*.

![Deactivating fragments](image)

**Figure 6: Examples of deactivating fragments**

The literature also describes sub-classes of alerts that represent cohorts of concern or highly active subclasses. These are essentially another alert; however, they can be linked with the other alerts through the hierarchical relationship as described earlier. For example the benzene, 1-amine(NH2)-, 4-aryl- is identified as a specific sub-class of the primary aromatic amines since “these chemical features make the nitrenium ion (DNA-reactive intermediate) more stable due to the electron donating property of the benzene ring, and thus more reactive with DNA” [35]. There are also limited examples in the literature so a data mining exercise of the reference set was undertaken to identify these classes.

The complex relationships between the alerts, and the deactivating fragments and/or highly active subclasses (all organized in a hierarchy) as well the accompanying non-structural information such as the source of the alerts and the mechanistic rationale for the alert is encoded as an XML document [36]. The alert can also be defined as one or more “Rules” to accommodate examples such as *polycyclic aromatic hydrocarbons*, which require the definition of a number of unique cyclic systems to fully define all possible planar systems for this alert. This XML document is linked to the structural definitions for the
alerts, deactivating fragments, and the highly active subclasses, which are defined in the SD file format [37]. The information contained in the XML file as well as the structural definitions, provides the source of the information used by the expert alerts system. Figure 7 provides an example of an XML alert record for an alert.

Figure 7: Example of an XML record to represent an alert

Alert Assessment

The purpose of the alert assessment process is to establish which alerts should be used to make predictions. These “active” alerts should have clear evidence that they would be predictive of a positive outcome in the bacterial mutagenesis assay, as required by the ICH M7 guideline. To make this assessment, alerts are initially assigned as active where there are more than five examples of compounds in the reference set that match the alert and when greater than 70% of those examples are positive. However, it is not enough to rely solely on the number of examples for each alert. A second assessment is performed by removing those compounds that also contain other alerts. Where compounds containing only the alert in question also show good correlation, then there is more convincing evidence for the alert to be assigned as active. This assessment is illustrated in Figure 8 with two examples. There is good supporting data to include the aziridine in the list of active alerts. Another example of a potential alert is furans, a proposed alert for mutagenicity from the paper [30]. There is some evidence of an association in the reference set with 143 examples since almost 66% were positive (compared to 47% active in the entire reference set). However, if compounds that contain an additional alert such as an aromatic nitro, etc. are removed, there are only 33 remaining examples from the reference set with 24% positive (lower than the average for the reference set). Hence, based upon this data there is not enough evidence to suggest furan should be included as an active alert.
Alerts are also classified as “inactive” for the bacterial mutagenesis assay. These alerts show little correlation with the data. In these cases a cut-off of five examples with less than 50% positive, which represents the approximate percentage of active/inactive in the entire reference set, is used.

There is a final category which is assigned to the remaining alerts – “indeterminate”. Alerts where there is insufficient number of examples to classify the alert as either active or inactive would fit into this category. Alternatively, there may be a sufficient number of examples, but, the number of positive examples is between the active and inactive cut-off (50% - 70% positives). One explanation is that there may be insufficient information that can be gleaned from the data concerning deactivating factors or highly active subclasses. Another group of indeterminate alerts exist when there is additional information to question the alert. For example, it has been reported that acid halides show positive Ames data only in the presence of and as a consequence of the use of DMSO [38]. Hence, this additional knowledge could be used to assign them as indeterminate. Although, indeterminate alerts are not be used to make any positive calls, they may be used in subsequent expert review. Since the alerts are hierarchically organized, it is possible to have a mixture of active, inactive and indeterminate alerts at various levels of the hierarchy which illustrates another benefit of this hierarchical organization.

**Classification and scoring**

To use alerts to make predictions to support a regulatory decision, i.e. whether a test compounds should be classified as positive or negative, it is necessary to devise a series of rules for classification. In addition, it is important to understand whether the compound is in the applicability domain and hence whether any prediction could be made at all.

A positive call is made for compounds within the applicability domain where an active alert matches the test compound and there are no deactivating fragments in the specified relationship to the primary alert. In Figure 9, the two chemicals presented would be predicted as positive. Chemical A matches a
terminal hydrazine (highlighted in red) and chemical B matched both an aromatic nitro as well as an aziridine.

![Figure 9: Examples of positive results](image)

<table>
<thead>
<tr>
<th>Call</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision</td>
<td>0.94</td>
</tr>
<tr>
<td>Alert fired</td>
<td>152: terminal hydrazine</td>
</tr>
</tbody>
</table>

Where an active alert matches, yet a deactivating fragment is also present in the portion of the structure as the active alert, then a negative call is made. A negative call is also made where no alert is identified and the test compound is within the applicability domain. In Figure 10, both chemicals C and D are predicted negative. In chemical C, an aromatic primary amine is present (shown in gray); however, there is a corresponding deactivating fragment in the 2-position. In chemical D, no alerts are identified. A prediction for both chemicals can be made because they are in the applicability domain of the alerts based on their distances to at least one compound in the reference set.

![Figure 10: Examples of negative results](image)

<table>
<thead>
<tr>
<th>Call</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Precision</td>
<td>0.1567</td>
</tr>
<tr>
<td>Alert fired</td>
<td>No Alerts</td>
</tr>
</tbody>
</table>
It is essential to only make a negative call for compounds that do not represent novel classes of chemistry where no historical data has been collected. This assessment of the applicability domain is made using a structural similarity cut-off to the reference set based on the Leadscope structural fingerprint and a Tanimoto distance [19-24]. This ensures that negative predictions are not extrapolated to novel areas of chemistry where there is no Ames data. A call of indeterminate occurs when only an alert marked as indeterminate is identified yet the compound is still in the domain of applicability. Figure 11 illustrates a not-in-domain result as well as an indeterminate result.

![Figure 11: Examples of out-of-domain and indeterminate results](image)

To accompany the positive or negative classification, a score is generated further indicate the confidence in the prediction. This score reflects the ratio of positive/negative example compounds from the reference set and is defined as precision. When multiple alerts match the test compound, the most precise or highest score is selected. When a negative classification is made, the score is reflective of the background positive/negative ratio for all reference set compounds with no active alerts. In Figure 9, chemical A matches a terminal hydrazine which is 94% positive based upon the examples in the database. Compound B in Figure 9 matched two alerts and the alert with the highest precision value is selected, in this case aziridine. In Figure 10 compound C and D both have a precision score of 0.1567 which is the positive/negative ratio for all compounds that do not contain an alert in the reference set.

**Consensus rules for ICH M7**

The ICH M7 guidance states that two *in silico* methodologies need to be used in the assessment of impurities; however, if data is available it can also be used. Thus a complex series of possibilities exist for generating an overall final call. Generally, the availability of data will take precedence over a prediction. If one or more prediction method indicates a positive, in the absence of data related the predicted endpoint, the overall call is positive.
Results
The Leadscope Ggenetox Expert Alerts (described in this paper) have been implemented as part of the Leadscope Model Applier v1.8 (alongside the existing statistical-based QSAR models). To assess the performance of this alert system, two data sets were used: (1) the reference set (as described in Reference set section) and (2) the Hansen data set. The Hansen set includes data described in the Hansen et al. publication [39] where the full set includes 6,512 chemicals. However, 2,680 were in the RCA-QSAR training set so were removed. A number of other chemicals were also removed based on stereochemical considerations or their inability to be modelled leaving 3734 compounds. Figure 12 shows the performance results for the reference set. A positive/negative prediction was made for 6,949 of the 7,112 total number of chemicals (97.9% coverage). There were 5,868 correct predictions or 84.1% concordance (2,749 true positive plus 3,119 true negative), with 528 false positives and 553 false negatives. The values for sensitivity, specificity, negative predictivity as well as positive predictivity are also presented. Figure 13 shows the corresponding performance statistics for the Hansen data set.

<p>| | |</p>
<table>
<thead>
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<tbody>
<tr>
<td>Concordance</td>
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<tr>
<td>Sensitivity</td>
<td>83.3%</td>
</tr>
<tr>
<td>Specificity</td>
<td>85.5%</td>
</tr>
<tr>
<td>Negative Predicitivity</td>
<td>84.3%</td>
</tr>
<tr>
<td>Positive Predicitivity</td>
<td>83.9%</td>
</tr>
<tr>
<td>Coverage</td>
<td>97.9%</td>
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</table>

Figure 12: Performance statistics (reference set)

<p>| | |</p>
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Concordance</td>
<td>79.6%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>87.0%</td>
</tr>
<tr>
<td>Specificity</td>
<td>69.3%</td>
</tr>
<tr>
<td>Negative Predicitivity</td>
<td>79.5%</td>
</tr>
<tr>
<td>Positive Predicitivity</td>
<td>79.6%</td>
</tr>
<tr>
<td>Coverage</td>
<td>98.6%</td>
</tr>
</tbody>
</table>

Figure 13: Performance statistics (Hansen)

In Figure 14, the performance of the alert system is compared to the performance of the M7 consensus call for the Hansen data set. This analysis also takes into account the availability of data as well as the
results from two statistical-based QSAR models for Salmonella and E. coli [10,11]. This M7 consensus call results in improved performance, particularly for sensitivity and negative predictivity.

<table>
<thead>
<tr>
<th></th>
<th>Hansen (alerts)</th>
<th>Hansen (M7 consensus)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>79.4%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>87.0%</td>
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<tr>
<td>Specificity</td>
<td>69.3%</td>
<td>58.4%</td>
</tr>
<tr>
<td>Negative Predictivity</td>
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<td>89.1%</td>
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<tr>
<td>Positive Predictivity</td>
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</tr>
<tr>
<td>Coverage</td>
<td>98.6%</td>
<td>99.7%</td>
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</tbody>
</table>

Figure 14: Performance statistics (M7 consensus for the Hansen set)

Discussion
ICH M7 guidance outlines for regulators and pharmaceutical sponsors the process for qualifying a drug impurity as having no mutagenicity concern. As part of this process a computational analysis can be performed to qualify certain classes of impurities as negative. The guideline also states that any in silico systems should adhere to the OECD validation principles where models should have: “1) a defined endpoint, 2) an unambiguous algorithm, 3) a defined domain of applicability, 4) appropriate measures of goodness-of-fit, robustness and predictivity, 5) a mechanistic interpretation, if possible.” The new expert alert-based system described here is built to specifically make predictions for the bacterial mutation endpoint following the ICH M7 guidance. The algorithm has been outlined in detail in this publication including the methodology used to assess the applicability domain. The results from both an internal and external validation have been presented which summarizes the system’s goodness-of-fit, robustness and predictivity. Finally, when any alert matches a target test compound, a summary of the literature-derived mechanistic basis for each matched alert is provided.

One of the guiding principles in the development of this system was transparency of the predictions. This is implemented by linking the alerts to a quantitative assessment based on an extensive collection of mutagenicity data. This not only provides a level of confidence in the results but also supports expert judgment that may accompany the results. The alerts are defined such that it is possible to immediately answer the questions – which literature sources did the matched alerts come from, how is the structure of the alert defined, what is their mechanistic justification, what data supports the use of the alert (see Figure 15). The system will also highlight the presence of any indeterminate alert and hence could be used as part of any additional expert opinion.
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The system also addresses how results from both an expert alert-based system in combination with statistical-based QSAR models as well as any available experimental data can be used to generate an overall call to use in the assessment of the ICH M7 compliant results. The ICH M7 guidance includes language stating that when an API has been tested and the results were negative, but an impurity was predicted positive and both the API and the impurity share a positive alerting fragment, then it is possible to argue that the impurity could be classified as negative. By highlighting the components of both the statistical-based model as well as the expert-alerts based model that trigger the positive results, it is possible to readily make this expert opinion, as illustrated in Figure 16.
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March 2014

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Figure 16: Looking at the weight of evidence

<table>
<thead>
<tr>
<th>Structure</th>
<th>M7 Consensus</th>
<th>E. Coli - Sal 102 A-T Mut Positive</th>
<th>E. Coli - Sal 102 A-T Mut Negative</th>
<th>Salmonella Mut Positive</th>
<th>Salmonella Mut Negative</th>
<th>Bacterial Mutation Experimental</th>
<th>Bacterial Mutation Matched Alerts</th>
<th>Bacterial Mutation Mutagenicity Alerts</th>
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<tbody>
<tr>
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<td>Negative</td>
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<td>LS-63262 (2)</td>
<td>Positive</td>
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</table>

Conclusion

A new expert alert-based system has been described to directly support the ICH M7 guideline for drug impurities. This paper has described the process of developing the alert knowledge base as well as the reference set used to quantitatively assess the alert rules. These alerts are based on well-defined mutagenicity structural alerts from the literature. They have been assessed, alongside deactivating factors as well as active subclasses (which represent possible cohorts of concern). The paper has also described how predictions are made based on the presence of an alert with no defined deactivating factors as well as determining whether the target test compound is similar enough to known classes of chemicals to be predicted - that it is not trying to extrapolate to areas of chemistry the system has never seen. The good validation results as well as its adherence with the ICH M7 guidance and OECD validation principles allow this system to be used in the assessment of impurities with confidence.

Acknowledgements

We would like to thank Naomi L. Kruhlak, Lidiya Stavitskaya, and Barbara L. Minnier from the US FDA who, through the RCA agreement have provided Leadscope with access to the RCA-QSAR data set as well as input into the system. We would also like to thank Errol Zeiger and Ron Snyder for their expert advice as well as the beta testers from the pharmaceutical industry and consultants to the pharmaceutical industry.
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