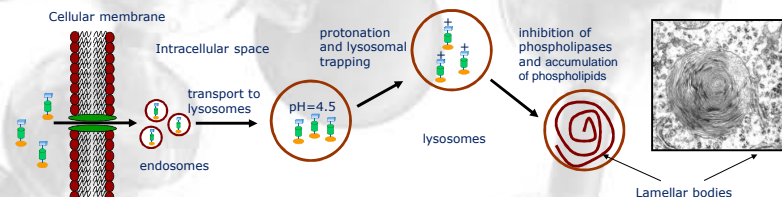


## Abstract

The loss of drug candidates during the later stages of pre-clinical development is a matter of concern to the pharmaceutical industry. One finding that threatens drug development is phospholipidosis, which is observed for many cationic amphiphilic drugs (CADs) during repeat-dose toxicity studies. Phospholipidosis (PLOSis) is an intracellular accumulation of phospholipids, often in lysosomes, manifested as lamellar bodies detected by electron microscopy (figure 1).



**Figure 1.** Mechanism for induction of phospholipidosis by CAD.

PLOSis may be an adaptive response and has not been unequivocally associated with toxic effects. However a PLOSis positive result could prevent further development of a drug candidate or prompt additional testing to prove that the effects observed in animals are not relevant to humans. To overcome this problem, a lot of effort was put into developing methods to predict potential ability of chemicals to induce PLOSis at an early stage of drug development.

## Introduction and aims

One of the first models for the prediction of phospholipidosis was published in 2004 [1] and since that time improvements have been suggested by several researchers [2, 3]. However the main structural features covered by these models were those of CADs, which do not describe all compounds that induce phospholipidosis. The work presented here shows the importance of evaluating data in the framework of a mechanistic rationale to derive new models for phospholipidosis from compounds previously poorly predicted by existing methods.

## Materials and methods

Initial investigations were conducted using non-proprietary data from an FDA phospholipidosis data set [4]. For the purpose of this poster the published phospholipidosis data set containing 185 compounds (102 active, 83 inactive) provided by Dr. J. Mitchell [5] was analysed. The authors derived this data set from two previously published papers [2, 4], but retained only compounds with histopathology or electron microscopy data. The supplied SMILES strings were converted into structures in JChem for Excel, imported into Instant JChem (both ChemAxon) and an SD file was exported. This file was processed against the phospholipidosis endpoint in Derek for Windows Version 12 (DfW12). Visual analysis of false negative compounds was used for identification of potential new classes for phospholipidosis. The published literature was searched to collect additional evidence to support a proposed SAR for each class.

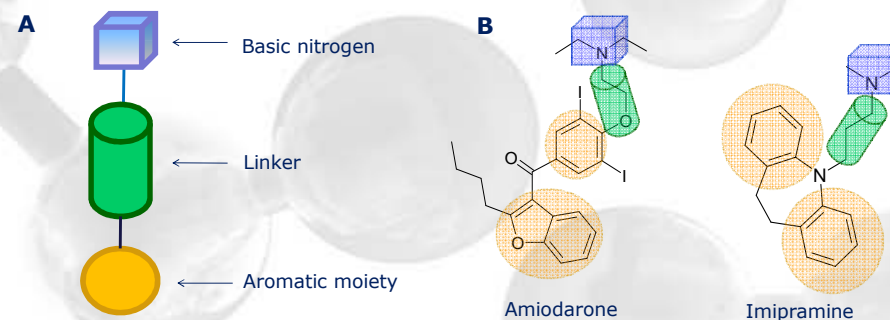
## References

1. Ploemen et al. *Exp Toxicol Pathol.* **2004**, 55, 347-355. 2. Pelletier et al. *J Chem Inf Model.* **2007**, 47, 1196-1205. 3. Hanumegowda et al. *Chem Res Toxicol.* **2010**, 23, 749-755. 4. Kruhlak et al. *Toxicol Mech Methods.* **2008**, 18, 217-227. 5. Lowe et al. *Mol Pharm.* **2010**, 7, 1708-1714. 6. Nagai and Takano. *Drug Metab Pharmacokin.* **2004**, 19, 159-170. 7. Anonymous. *Physicians' Desk Reference*, 59th edition, Thomson PDR, Montvale, **2005**. 8. Whitehouse et al, *Toxicology*, **1994**, 94, 81-95.

## Results

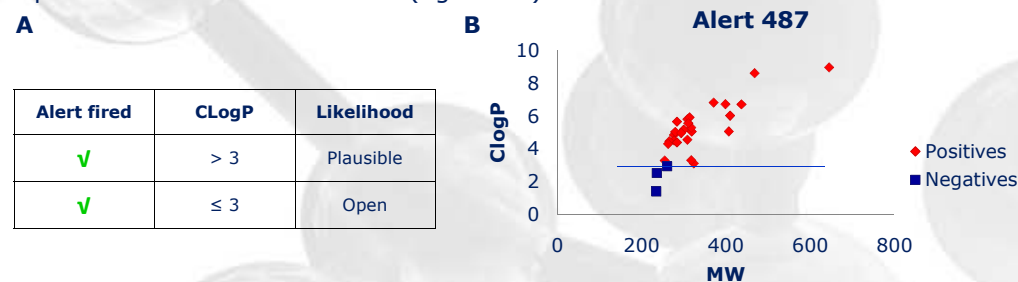
### 1. Predictive performance of existing structural alert for phospholipidosis

DfW12 contains a single structural alert for phospholipidosis (487). This alert is based on the structural features of CADs, namely a basic nitrogen connected via a linker to an aromatic moiety. A schematic representation of the pharmacophore is given in figure 2.



**Figure 2.** Phospholipidosis alert 487. A - Schematic representation of pharmacophore, B - representative examples supporting the alert.

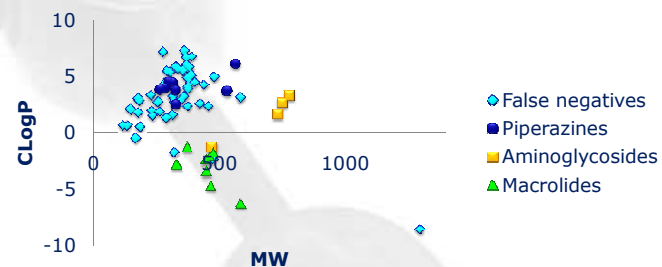
The overall result for this structural alert also incorporates elements of Ploemen's rule [1], i.e. compounds where  $[(\text{CLogP})^{-2} + (\text{pK}_a)^{-2}] > 90$  are expected to induce phospholipidosis, if  $\text{CLogP} > 1$  and  $\text{pK}_a > 8$ . Since the vast majority of compounds activating the structural alert had a  $\text{pK}_a$  of approximately 9, the rule was simplified and implemented in the knowledge based as a CLogP-thresholded rule (figure 3A). In this way, predictions are refined, allowing discrimination between positive and negative compounds that activate the alert (figure 3B).



**Figure 3.** Compounds that activate alert 487. A - ClogP-threshold rule, B - distribution of chemicals CLogP/MW.

### 2. Analysis of false negatives

Visual analysis of false negative compounds led to identification of three potential chemical classes for phospholipidosis: piperazines, aminoglycosides and erythromycin-like macrolides. Plotting CLogP values versus MW showed that the last two classes occupy significantly different chemical space to the rest of false negative compounds (figure 4).



**Figure 4.** Distribution of false negative compounds.

### 3. New chemical classes for phospholipidosis

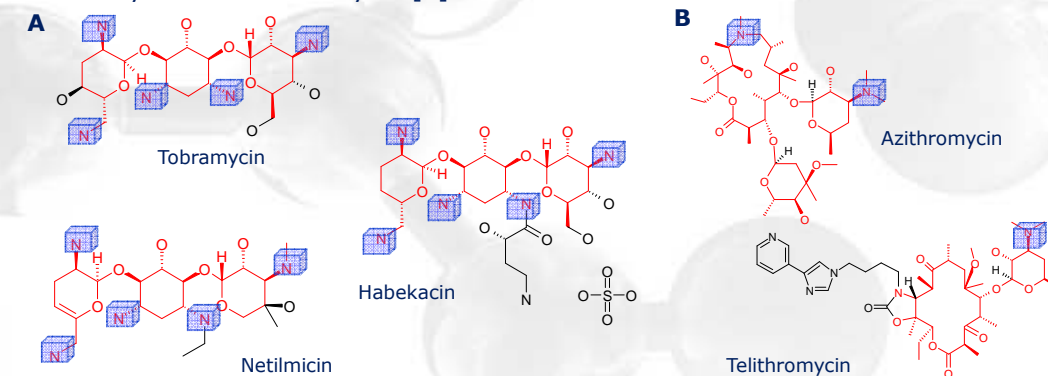
All three classes were researched and supporting data on the phospholipidosis induced by these chemicals were collected.

#### 3.1. Structural classes occupying different chemical space to CADs

Although aminoglycosides and macrolides are structurally dissimilar to CADs, they still contain basic nitrogen atoms (a feature of phospholipidosis-positive CADs). Representative compounds for each class are shown in figure 5, with the toxicophore highlighted in red.

Aminoglycosides are reported to accumulate in renal proximal tubule cells facilitated by megalin, a cell-surface protein that is highly expressed in these cells [6], followed by

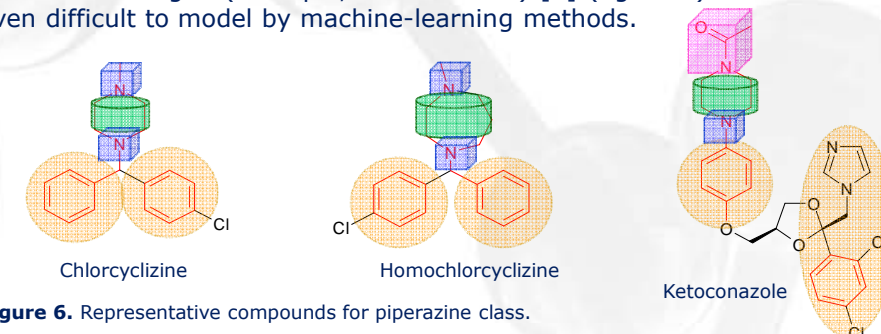
concentration in lysosome where they inhibit phospholipases and sphingomyelinase, leading to increased in lysosomal phospholipid levels. For macrolides the mechanism of phospholipidosis is not clear, but lamellar bodies were observed in laboratory animals treated with high doses of erythromycin, azithromycin and telithromycin [7].



**Figure 5.** Representative compounds for new chemical classes for phospholipidosis (toxicophores are highlighted in red): A - aminoglycosides, B - macrolides.

#### 3.2. False negatives structurally similar to CADs

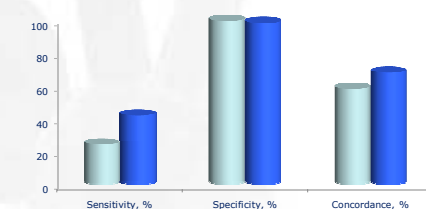
Several false negatives were found to contain a piperazine ring, and these occupy similar chemical space to CADs. Though similar, these compounds have two distinct features: a second basic amine group (present in the piperazine ring) and a requirement for a second aromatic ring in order for phospholipidosis to be observed. Additionally this alert includes compounds which can be metabolised to give a basic nitrogen (example, ketoconazole) [8] (figure 6). Such metabolism has proven difficult to model by machine-learning methods.



**Figure 6.** Representative compounds for piperazine class.

#### 4. Predictive performance of new alerts for phospholipidosis

Implementation of new alerts for phospholipidosis are projected to lead to improvements to predictive performance nearly doubling sensitivity, with a concomitant increase in concordance and only marginal decrease in specificity (figure 7).



**Figure 7.** Projected predictive performance.

#### 5. Future work

Whilst this work should improve the predictive performance of DfW for phospholipidosis, there are still a number of false negative compounds from the Low data set. Future plans involve the further investigation of the CAD-like chemical space around alert 487. It is possible that loosening the structural restrictions around this alert, whilst including a  $\text{pK}_a$  assessment in the reasoning, could lead to additional increases in sensitivity. Further, analysis of both the proprietary and non-proprietary data held by the FDA should also enable alert development.

## Conclusions

An expert evaluation of data for compounds causing phospholipidosis allowed the following:

- implementation of alerts for compounds that are structurally different from CADs, which are difficult to predict by ML methods
- an understanding of the mechanism underlying phospholipidosis, which can increase the confidence in, and transparency of, a prediction
- evaluation of multiple data sources, such as the metabolism data for ketoconazole that were used to define the scope of the structural alert for piperazines