Development of a decision tree for mitochondrial dysfunction: Uncoupling of oxidative phosphorylation

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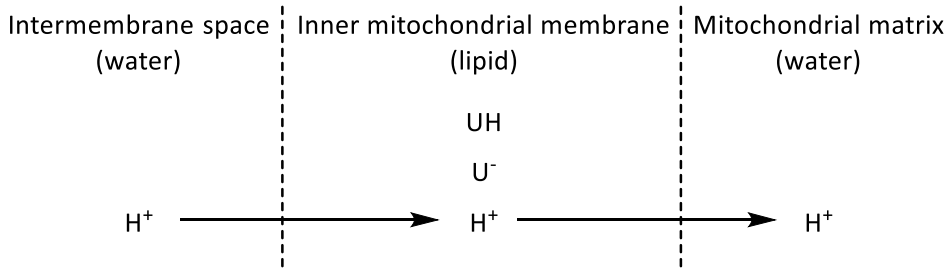


Abstract

Mitochondrial dysfunction is the result of a number of processes including the uncoupling of oxidative phosphorylation. This study outlines the development of a decision tree-based profiling scheme capable of assigning chemicals to one of six confidence-based categories. The decision tree is based on a set of structural alerts and physico-chemical boundaries identified from a detailed study of the literature. The physico-chemical boundaries define a chemical relationship with both log P and pKa. The study also outlines how the decision tree can be used to profile databases through an analysis of the publically available databases in the OECD QSAR Toolbox. This analysis enabled a set of additional structural alerts to be identified that are of concern for protonophoric ability. The decision tree will be incorporated in the OECD QSAR Toolbox V4.3. The intended usage being for the grouping of chemicals into categories for chronic human health and environmental toxicological endpoints.

Keywords: uncoupling of oxidative phosphorylation, structural alerts, parametric boundaries, decision tree

Introduction

The mitochondria play an important role in a range of processes vital to normal cellular function, key amongst these being the production of approximately 95% of the ATP generated during oxidative phosphorylation. The structure of the mitochondria consists of two membranes, the outer and inner membrane, enclosing three compartments, the inter-membrane space, the cristae and the mitochondrial matrix. It is across the inner mitochondrial membrane (IMM) that the process of oxidative phosphorylation occurs. Briefly, this process involves the transfer of electrons down the electron transport chain in conjunction with the pumping of protons from the mitochondrial matrix into the inter-membrane space. The movement of these protons creates an electrochemical gradient across the IMM, which is utilised by ATP-synthase to convert adenosine diphosphate into adenosine triphosphate [1, 2]. Given the importance of this process, it is of no surprise that chemicals that can disrupt it are of concern in both human and environmental toxicology.

The simplest protonophoric mechanism involves the anionic conjugate base partitioning into the IMM from the intermembrane space. Once within the IMM the anion collects a proton at the IMM -water interface. The now protonated acid migrates across the IMM to the IMM -water interface on the matrix side. At this interface the proton is released into the mitochondrial matrix. The regenerated anion remains in the IMM enabling it to repeat the cycle [3]. This mechanistic hypothesis has been extended, with cyclic voltammetry data showing that both the acid and its conjugate base need to be present in the IMM in order for the observed bell-shaped dependency between pH and protonophoric activity to be accounted for [4, 5]. This mechanistic hypothesis is summarised in Figure 1.

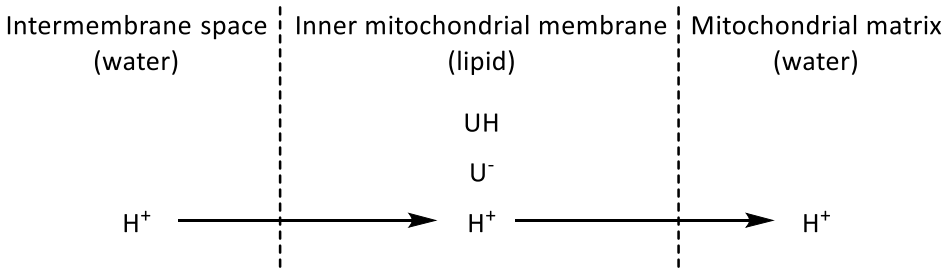


Figure 1: Potential mechanism for protonophoric uncoupling of oxidative phosphorylation (the dashed lines represent the membrane-water interfaces)

A number of weak acids have been reported as causing uncoupling of oxidative phosphorylation as they are able to transport protons across the IMM. There have been two key sets of structural alerts published for mitochondrial dysfunction, including protonophores [6, 7]. The primary set being from the work of Naven and co-workers who identified 14 potential alerts for protonophores from a dataset of 2085 chemicals tested in the respiratory screening technology (RST) assay [7]. Of these alerts, 11 were subsequently incorporated into an *in silico* screening tool for which the positive predictivity was 83% and a negative predictivity of 97%. A more recent study analyzed a smaller set of 288 chemicals identifying a total of 17 structural alerts related to mitochondrial dysfunction in general [6]. Of these alerts, 14 were related to protonophores, of which four had not been previously reported in the work of Naven et al. Outside of these two key publications the remaining structure-activity knowledge for protonophores is located in the numerous quantitative structure-activity relationships models that have been constructed for the prediction of uncoupling of oxidative phosphorylation in both human and environmental toxicity [6-11, 6-8, 12, 13, 6, 14, 15].

It is the aim of this study to develop a comprehensive set of structural alerts derived from literature data sources. These structural alerts, augmented with physico-chemical property boundaries are incorporated into a decision tree capable of assigning chemicals to one of three categories of concern for uncoupling of oxidative phosphorylation.

Methods

A set of 2D structural alerts are defined based on literature reports of protonophoric activity in mitochondria (see Table 2 for details). The alerts were encoded into the OECD QSAR Toolbox as part of a decision tree-based profiling scheme. In addition, relevant physico-chemical properties in terms of hydrophobicity and acid ionization were identified as appropriate quantifications of boundaries. Since structure-activity for protonophoric activity is related to the ability of a chemical to reside within the IMM [4], membrane partitioning quantified by the 1-octanol/water partition coefficient (log P) can be used to establish a minimal hydrophobic boundary for entry into the decision tree.

Based on two facts, pKa is also a logical property for setting boundaries within the protonophores decision tree. First, general structure-activity for protonophoric activity is related to the ability of an acidic proton to be partially dissociated within the IMM; thus, enabling protons to be shuttled back into the matrix [4]. Second, pKa values for enhanced protonophoric behavior have been suggested to be between 5.3 and 6.3 [16], with the vast majority of protonophores having pKa values between 3.0 and 9.0 [17].

The rationale for using pKa values in establishing boundary constraints, require some comment in regards to the impact of pKa on ionization in different biological media as the degree of ionization is dependent on the solvent [4]. It is important to note that pKa values quoted in the literature are determined in an aqueous environment, typically at 25˚C. It has been shown that the equivalent pKa values in the IMM are approximately 1.0 log unit higher [4]. For example, an acid with a pKa value of 3.0 will be completely ionized at the pH values found in the mitochondria (i.e., around 7.0 in the inner membrane space and 8.0 in the mitochondrial matrix [18, 19]). This is important when one considers the need for a chemical to remain partially ionized in the IMM in order to efficiently act as a protonophore. The information in Table 1 leads to the premise that the most potent protonophores are chemicals with aqueous pKa values of around 6.0; their pKa values in the membrane are closer to 7.0 making ≈ 50% of the membrane-contained acid ionized. More specifically, this premise is supported when one considers that 2-[[4-(trifluoromethoxy)phenyl]hydrazinylidene]propanedinitrile or FCCP (pKa = 6.2) is 1000 times a more potent protonophore than 2,4-dinitrophenol (pKa = 4.1) [20]. All pKa values were calculated with ChemAxon pKa calculator V5.11. Chemical structures and names were produced using ChemDraw professional V16.0.1.4

Table 1: Variation in the % ionization in an aqueous and lipid environment at pH 7.0.

$pKa_{aqueous}$	% ionization water	$pKa_{mito\text{-}membrane}$	% ionization lipid
3.0	99.99	4.0	99.90
4.0	99.90	5.0	99.00
5.0	99.00	6.0	90.91
6.0	90.91	7.0	50.0
7.0	50.00	8.0	9.09
8.0	9.09	9.0	0.99
9.0	0.99	10.0	0.09

Results and Discussion

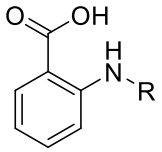
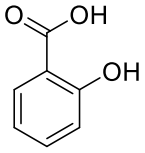
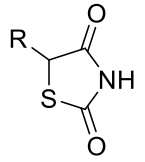
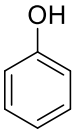
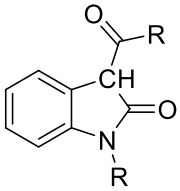
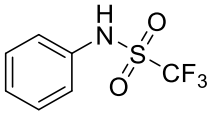
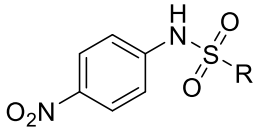
Here is reported the details of structural alerts, parametric boundaries and related *in silico* decision tree for mitochondrial dysfunction due to the action of protonophores. This decision tree has been

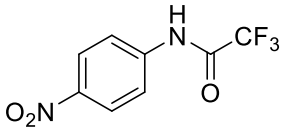
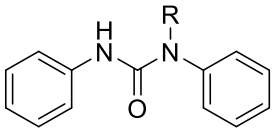
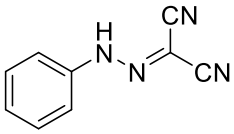
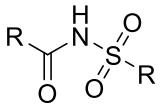
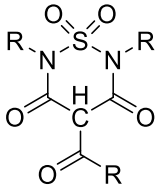
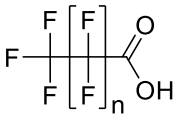
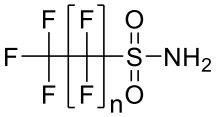
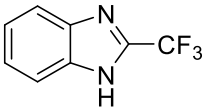
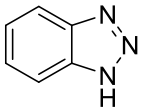
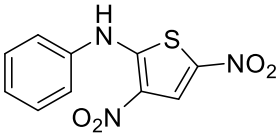
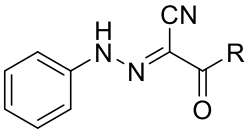
implemented within the OECD QSAR Toolbox, profiling of the databases within this system demonstrate the utility of the approach.

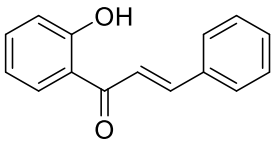
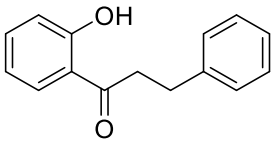
Structural alerts

A set of 2D structural alerts were defined based on literature reports of protonophoric activity in mitochondria (Table 2). The structural boundaries of the alerts show that 18 out of 20 alerts contain an acidic proton attached to either an oxygen or nitrogen atom. The exceptions are alerts 5 and 12 which contain an acidic proton attached to a carbon atom.

Table 2: Initial set of structural alerts identified from the literature suggested to be associated with protonophoric activity in the mitochondria (N.B. all rings can be substituted or part of a larger structure, R and n denote the substituent or chain length used to calculate the shown pKa value). Alert names are as detailed in the original literature sources.

ID	Name	Structural alert	pKa	References
1	Anthranilic acids	 R = any hydrogen or carbon	4.9 (R = Me)	6-11
2	Salicylic acids		2.8	6, 7, 9
3	Thiazolinediones	 R = any hydrogen or carbon	6.6 (R = Me)	6, 7, 9
4	Phenols		10.0	6-9, 11, 13
5	Aclindolones	 R = any hydrogen or carbon	7.5 (R = Me)	6, 7
6	Fluoromethylsulphonanilides		3.9	6, 7
7	Nitrosulphonanilides	 R = any carbon	8.1	6, 7

8	Trifluoroacetamido Nitrobenzenes		10.4	6, 7
9	Diphenyl ureas	 R = any carbon	11.8 (R = Me)	6, 7
10	Carbonylcyanide phenylhydrazones		6.3	6-8, 12, 13
11	Acyl sulphonamides	 R = any hydrogen or carbon	4.1 (R = Me)	6, 7
12	Thiadiazinedione dioxides	 R = any hydrogen or carbon	-0.6 (R = Me)	6, 7
13	Perfluorinated carboxylic acids (PFOA)	 n = 5 – 11	1.4 (n = 5)	6, 14
14	Perfluorinated sulphonamides (PFOSA)	 n = 5 – 11	3.4 (n = 5)	6, 14
15	2-Trifluoromethyl benzimidazoles		9.9	8, 9, 11, 21
16	Benztriozoles		8.6	8
17	Phenylaminodinitrothiophenes		8.7	8
18	α-Acyl-α-cyano-carbonylphenylhydrazones	 R = any hydrogen or carbon	6.1 (R = Me)	8

19	2-Hydroxychalcones		7.2	[15]
20	2-Hydroxy dihydrochalcones		9.1	[15]

Parametric boundaries

A log P-based cutoff and four pKa-bounded chemical spaces were identified (Table 3). Subsequently, these physico-chemical boundaries were aligned to levels of concern that apply to all of the alerts (detailed in Table 2).

Table 3: Physico-chemical parametric boundaries for levels of concern for protonophoric activity

Parameter	Value	Level of concern
Log P	< 1.5	No concern for protonophoric activity
Log P	≥ 1.5	Protonophoric activity increases above this minimum
pKa	≥ 5 and ≤ 7	High concern for protonophoric activity
pKa	≥ 3 and < 5	Moderate concern for protonophoric activity
pKa	> 7 and ≤ 8	Moderate concern for protonophoric activity
pKa	< 3 or > 8	No concern for protonophoric activity

Inspection of Table 2 shows that, as expected, the majority of the structural alerts identified from the literature (all of which have been reported to act as protonophores) have pKa values that lie within the boundaries defined in Table 3. It is worth noting that the pKa values listed are for the unsubstituted alert and as such these values will be affected by the nature of any substituents. For example, the pKa of 2-nitrophenol is 6.4 making it a protonophore of high concern in the mitochondria (compared to phenol for which the pKa value is 10.0). Thus, the inclusion of unsubstituted alerts that have pKa values greater than 8.0 is to be expected and highlights the importance of parametric boundaries in the decision tree. The use of such boundaries negates the need to define all possible substituents for each alert. In a similar fashion, salicylic acids (unsubstituted alert, pKa = 2.8 - alert 2 in Table 2) that are substituted with electron-donating groups (such as amide) are predicted to be of moderate concern due to their pKa values being greater than 3.0. As with structural alerts containing protonophores with pKa values greater than 8.0, this analysis further supports the importance of defining physico-chemical boundaries associated with the structural alerts.

There are four reported structural alerts for which the unsubstituted pKa values outside of the ranges typically associated with mitochondrial toxicity (i.e., Alerts 8, 9, 12 and 13 in Table 2). The structural alert for diphenyl ureas (Alert 8) and trifluoroacetamidonitrobenzenes (Alert 9) have pKa values of 10.4 and 11.8 respectively making them almost completely unionized in the mitochondria. At the other end of the pKa boundaries the pKa values for thiadiazinedione dioxides (Alert 12, pKa = -0.6) and perfluorinated carboxylic acids (Alert 13, pKa = 1.4) indicate them to be strong acids (meaning that they will be fully ionized anywhere within the cellular environment). It is worth noting that high concentrations of perfluorinated carboxylic acids are required to cause mitochondrial dysfunction [22]. Such high concentrations enable the unionized acid to exist in sufficient quantities for protonophoric

activity to occur. It is therefore possible, that a similar explanation could be used to rationalize the mitochondrial toxicity of alerts 8, 9 and 12.

Further inspection of the structural alerts listed in Table 2 reveals the pKa values of the ranges listed in Table 3 are not necessarily sufficient to assign the proper level of concern. Specifically, an important additional factor - the degree to which the negative charge is delocalized is identified. For example, simple aromatic carboxylic acids (pKa = 4.1) have been reported as being relatively poor protonophores [23], whereas anthranilic, and salicylic acids (Alerts 1 and 2, respectively, in Table 2) have been reported as acting as potent protonophores [7, 24].

These latter observations have been rationalized in terms of the appropriate ionized conjugate-bases being stabilized by intramolecular H-bonding (Figure 2); thus, enabling it to partition into the IMM more readily [3].

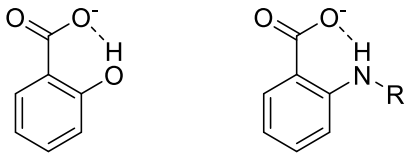


Figure 2: Potential intramolecular H-bonding present in the conjugate bases of anthranilic and salicylic acids (Alerts 1 and 2, respectively in Table 2)

A similar delocalization argument has been applied to the effect of resonance stabilization on a chemical's ability to partition into the lipid bi-layer. This is exemplified by comparing the high potency protonophore 2,4-dinitrophenol to the non-protonophore benzoic acid. Both substances have similar pKa values (pKa values of 4.0 and 4.1 respectively) [23]. However, the delocalization via potential resonance forms are markedly different (Figure 3).

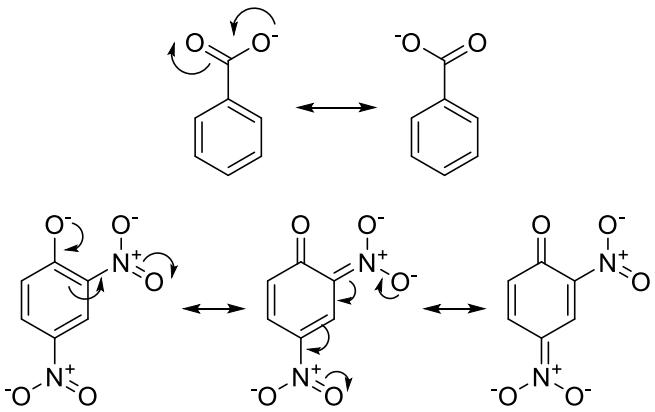


Figure 3: Delocalization through resonance of the negative charge present in the conjugate bases of benzoic acid (upper row of structures) and 2,4-dinitrophenol (lower row of structures)

Decision tree implementation

The structural alerts and parametric boundaries defined above were implemented into a decision tree (Figure 4). This decision tree provides a means of assigning chemicals to one of four categories with varying levels of confidence. The first of these categories is for chemicals that do not meet the hydrophobicity and acid ionization constant parametric boundaries i.e. chemicals that are unable to act as protonophores and/or partition into the inner mitochondrial membrane. This is followed by two

highly reliable categories for chemicals that are hydrophobic and fall either within the inner pKa range or, secondly, the outer pKa range. Chemicals assigned to either of these two categories are considered highly reliable as they also trigger a structural alert for which there is literature evidence relating the alert to uncoupling of oxidative phosphorylation. The final category is for chemicals that meet the parametric boundaries but do not trigger a structural alert. Chemicals in this category are considered to be of lower reliability due to the absence of any supporting literature linking any potential protonophore to mitochondrial dysfunction. It is worth noting that this does not mean that chemicals in this category should be ignored; rather they are potential new classes of protonophores that could be investigated further for their ability to uncouple oxidative phosphorylation. For example, confirmation of their protonophoric activity through appropriate *in vitro* experiments. The decision tree is incorporated in the OECD QSAR Toolbox V4.3.

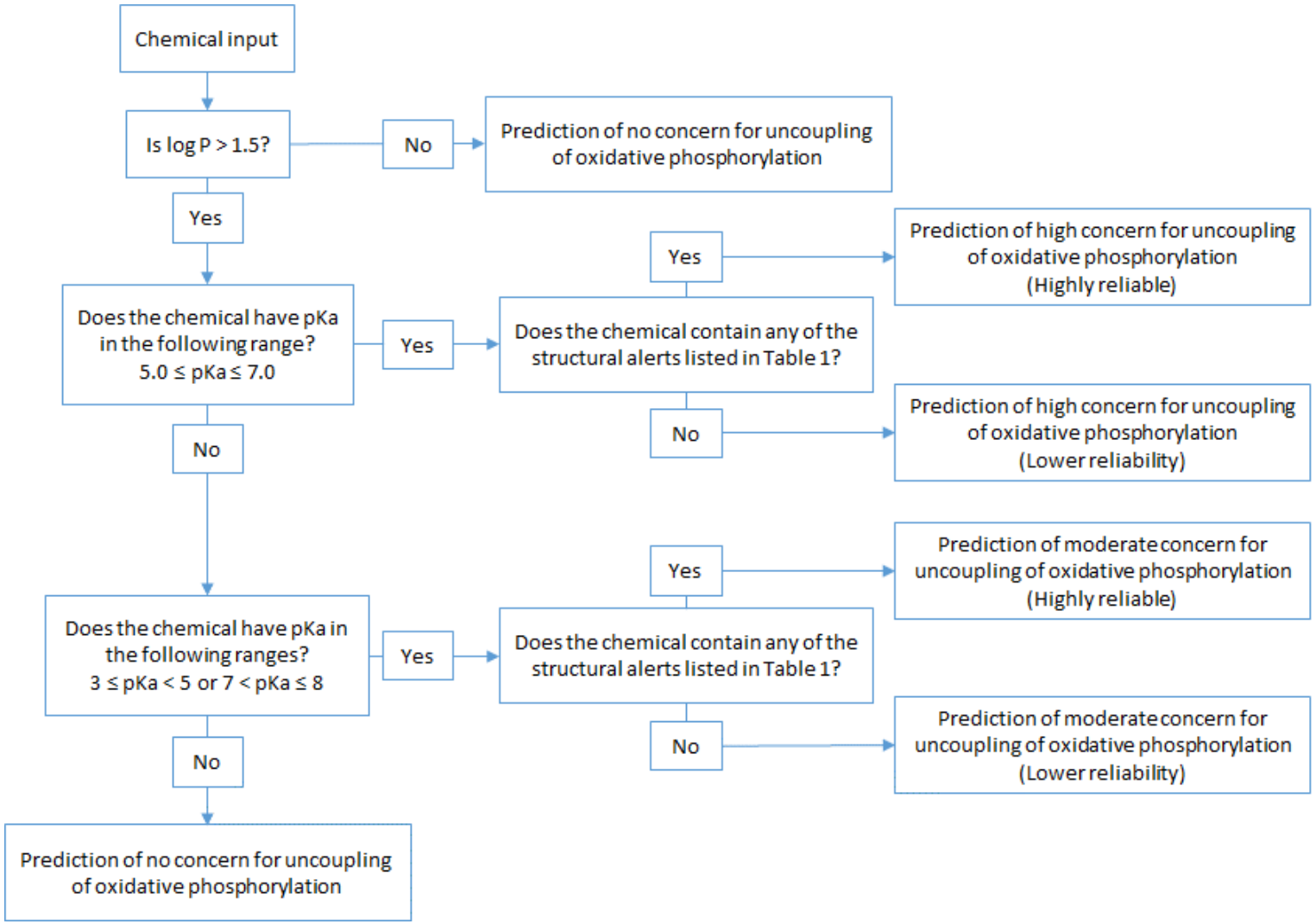


Figure 4: Decision tree implemented into the OECD QSAR Toolbox V4.3 for mitochondrial dysfunction caused by protonophore

Profiling results and additional alert development

As outlined, the structural alerts in Table 2 and the associated physico-chemical parameters were implemented into a decision tree in the OECD QSAR Toolbox (see Figure 4). This decision tree was used to profile the 31778 publically available chemicals in the Toolbox. Of these, 14703 fell outside the initial log P and molecular weight boundaries of the decision tree (having either a log P less than 1.5 or a molecular weight in excess of 1000g/mol). These chemicals were assigned to the non-uncoupling category in the decision tree. A further 12926 chemicals were also assigned to the non-uncoupling category based on their pKa values being outside of the ranges defined in Table 3. The remaining 4149 chemicals were identified as having the potential to act as protonophores, with 738 of these having a high concern for uncoupling activity (i.e. having a pKa value between 5 and 7). The final step in the decision tree relates to the presence of a structural alert related to protonophoric activity within the chemicals that fall within the pKa ranges. Of the 738 chemicals assigned as being of high concern a total of 306 were placed into the highly reliable category. Similarly, 662 chemicals of the 3411 chemicals of moderate concern were also placed into the highly reliable category. These profiling results are summarized in Table 4.

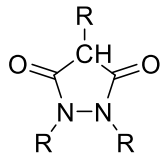
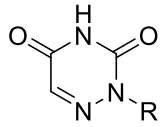
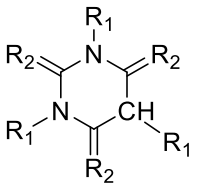
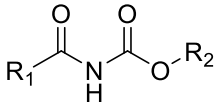
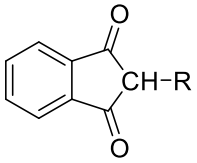
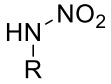
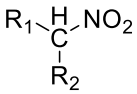
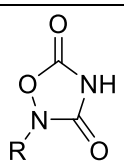
Table 4: Protonophoric profiling results based on the application decision tree in Figure 3 to the publically available databases in the OECD QSAR Toolbox

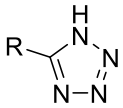
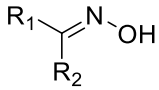
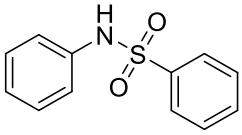
Category	Number of chemicals
MW > 1000 g/mol and/or log P < 1.5	14703
pKa < 3.0 or pKa > 8.0	12926
5 ≤ pKa ≤ 7 (high concern)	738
5 ≤ pKa ≤ 7 (high concern & highly reliable)	306
5 ≤ pKa ≤ 7 (high concern & lower reliability)	432
3 ≤ pKa < 5 or 7 < pKa ≤ 8 (moderate concern)	3411
3 ≤ pKa < 5 or 7 < pKa ≤ 8 (moderate concern & highly reliable)	662
3 ≤ pKa < 5 or 7 < pKa ≤ 8 (moderate concern & lower reliability)	2749

An analysis of the 432 chemicals assigned to the high concern & lower reliability category was undertaken in order to identify potential new structural alerts related to protonophoric activity. Table 5 shows the results of this analysis in which an additional 12 potential structural alerts were identified. Theses alerts meet the previously discussed parametric boundaries and, importantly, the conjugate base is likely to be sufficiently stabilized via resonance in order for it to partition into the mitochondrial lipid bi-layer. As with the structural alerts outlined in Table 2 these alerts feature acidic protons attached to either a nitrogen (four alerts) or carbon atom (five alerts). In addition, a structural alert for thiophenols involving an acidic proton attached to a sulfur atom was also identified.

As noted in Table 5, the majority of the unsubstituted alerts have pKa values between 3.0 and 8.0, the exception being structural New Alerts 6 and 11 (pKa values of 8.2 and 8.5, respectively). As with the OH group in the phenol alert (Table 2, Alert 4), the acidity of the NH group in New Alert 6 and the OH group in New Alert 11 (when R_1 is an aromatic ring) are greatly influenced by any electron-withdrawing groups attached to the aromatic ring. For example, the addition of an NO_2 group in the *para*-position reduces the pKa value of New Alert 6 to 6.8, placing such chemicals into the high concern category, justifying its inclusion as a potential new protonophoric alert.

Table 5: Potential new protonophoric structural alerts assigned to the low confidence category in the decision tree (alert names derived using ChemDraw professional, R, R_1 and R_2 indicate the substituents used to calculate the shown pKa value)

ID	Name	Structure	Number of chemicals	pKa
1	Pyrazolidine-3,5-diones	 R = any atom	8	pKa = 5.8 (R = H)
2	1,2,4-Triazine-3,5-diones	 R = any atom	1	pKa = 6.9 (R = H)
3	Pyrimidine-2,4,6-triones	 R_1 = any atom R_2 = oxygen or Sulphur	7	pKa = 5.0 (R_1 = H, R_2 = O)
4	Benzoylcarbamates	 R_1 = aromatic carbon R_2 = any carbon	2	pKa = 6.7 (R_1 = Ph, R_2 = Me)
5	2-Phenyl-indene-1,3-diones	 R = aromatic carbon	7	pKa = 4.9 (R = Ph)
6	*N*-Phenylnitramides	 R = aromatic carbon	2	pKa = 8.2 (R = Ph)
7	(Nitromethyl)benzenes	 R_1 = any atom R_2 = aromatic carbon	17	pKa = 6.1 (R_1 = H, R_2 = Ph)
8	1,2,4-Oxadiazolidine-3,5-diones	 R = any atom	1	pKa = 5.9 (R = H)
9	Benzenethiols	 R = aromatic carbon	47	pKa = 6.6 (R = Ph)

10	1*H*-Tetrazoles	 R = any atom	2	pKa = 6.0 (R = H)
11	Benzaldehyde oximes	 R_1 = sp^2 carbon R_2 = any atom	14	pKa = 8.5 (R_1 = Ph, R_2 = H)
12	Biaryl sulphonamides		44	7.9

Conclusions

This aim of this study was to develop a profiling scheme for chemicals capable of uncoupling oxidative phosphorylation in the mitochondria due to their ability to act as protonophores. An analysis of the literature identified a set of 20 structural alerts and five physico-chemical parametric boundaries in terms of log P and pKa associated with protonophoric behavior. These alerts and parametric boundaries were implemented into a decision tree-based profiling scheme within the OECD QSAR Toolbox. This scheme is able to assign a chemical to one of six categories with associated levels of confidence. The utility of this decision tree was demonstrated via the profiling of the publically available databases in the OECD QSAR Toolbox. This analysis enabled a further 12 new structural alerts to be identified with the potential to act as protonophores. The implemented decision tree is likely to be of use in chemical grouping and read-across predictions for chronic toxicity driven by mitochondrial dysfunction caused by protonophores.

References

(1) Wallace, K. B., and Starkov, A. A. (2000) Mitochondrial targets of drug toxicity. *Ann. Rev.Pharmacol. Toxicol. 40*, 353-388.

(2) Dykens, J. A., and Will, Y. (2008) *Drug-induced mitochondrial dsyfunction*. Wiley-Interscience, New Jersey, USA.

(3) Terada, H. (1990) Uncouplers of oxidative phosphorylation. *Environ. Health Perspect. 87*, 213-218.

(4) Ozaki, S., Kano, K., and Shirai, O. (2008) Electrochemical elucidation on the mechanism of uncoupling caused by hydrophobic weak acids. *Phys. Chem. Chem. Phys. 10*, 4449-4455.

(5) Ozaki, S., Shirai, O., Kihara, S., and Kano, K. (2007) Voltammetric study on the mechanism of ion transfer caused by weak acids in the bilayer lipid membrane. *Electrochem. Comm. 9*, 2266-2270.

(6) Nelms, M. D., Mellor, C. L., Cronin, M. T., Madden, J. C., and Enoch, S. J. (2015) Development of an in silico profiler for mitochondrial toxicity. *Chem. Res. Toxicol. 28*, 1891-1902.

(7) Naven, R. T., Swiss, R., Klug-Mcleod, J., Will, Y., and Greene, N. (2013) The development of structure-activity relationships for mitochondrial dysfunction: Uncoupling of oxidative phosphorylation. *Toxicol. Sci. 131*, 271-278.

(8) Buchel, K. H., and Draber, W. (1974) Structure—Activity Correlations of Acaricidal Hydrazones—Uncouplers of Oxidative Phosphorylation. *Adv. Chem. 114*, 141-154.

(9) Spycher, S., Smejtek, P., Netzeva, T. I., and Escher, B. I. (2008) Toward a class-independent quantitative structure-activity relationship model for uncouplers of oxidative phosphorylation. *Chem. Res. Toxicol. 21*, 911-927.

(10) Terada, H., Goto, S., Yamamoto, K., Takeuchi, I., Hamada, Y., and Miyake, K. (1988) Structural requirements of salicylanilides for uncoupling activity in mitochondria: quantitatvie analysis of structure-uncoupling relationships. *Biochem. Biophys. Acta 936*, 504-512.

(11) Tollenaere, J. P. (1973) Structure-activity relationships of three groups of uncouplers of oxidative phosphorylation: salicylanilides, 2-trifluoromethylbenzimidazoles, and phenols. *J. Med. Chem. 16*, 791-796.

(12) Balaz, S., Sturdik, E., Durcova, E., Antalik, M., and Sulo, P. (1986) Quantitative structure-activity relationship of carbonylcyanide phenylhydrazones as uncouplers of mitochondrial oxidative phosphorylation. *Biochem. Biophys. Acta 851*, 93-98.

(13) Miyoshi, H., Nishioka, T., and Fujita, T. (1987) Quantitative relationship between protonophoric and uncoupling activities of substituted phenols. *Biochem. Biophys. Acta 891*, 194-204.

(14) Wallace, K. B., Kissling, G. E., Melnick, R. L., and Blystone, C. R. (2013) Structure-activity relationships for perfluoroalkane-induced in vitro interference with rat liver mitochondrial respiration. *Toxicol. Lett. 222*, 257-264.

(15) Ravanel, P., Tissut, M., and Douce, R. (1982) Uncoupling activities of chalcones and dihydrochalcones on isolated mitochondria from potato tubers and mung bean hypocotyls. *Phytochemistry 21*, 2845-2850.

(16) Miyoshi, H., Tsujishita, H., Tokutake, N., and Fujita, T. (1990) Quantitative analysis of uncoupling activity of substituted phenols with a physicochemical substituent and molecular parameters *Biochim. Biophys. Acta (BBA) - Bioenergetics 1016*, 99-106.

(17) Wilson, D. F., Ting, P., and Koppelman, M. S. (1971) Mechanism of action of uncouplers of oxidative phosphorylation. *Biochemistry 10*, 2897-2902.

(18) Bal, W., Kurowska, E., and Maret, W. (2012) The final frontier of pH and the undiscovered country beyond. *PLoS One 7*, e45832.

(19) Santo-Domingo, J., and Demaurex, N. (2012) Perspectives on: SGP symposium on mitochondrial physiology and medicine: the renaissance of mitochondrial pH. *J. Gen. Physiol. 139*, 415-423.

(20) Childress, E. S., Alexopoulos, S. J., Hoehn, K. L., and Santos, W. L. (2017) Small Molecule Mitochondrial Uncouplers and Their Therapeutic Potential. *J. Med. Chem*. *61,* 4641-4655

(21) Jones, O. T. G., and Watson, W. A. (1967) Properties of substituted 2-trifluoromethylbenzimidazoles as uncouplers of oxidative phosphorylation. *Biochem. J. 102*, 564-573.

(22) Starkov, A. A., and Wallace, K. B. (2002) Structural determinants of fluorochemical-induced mitochondrial dysfunction. *Toxicol. Sci. 66*, 244-252.

(23) Lou, P. H., Hansen, B. S., Olsen, P. H., Tullin, S., Murphy, M. P., and Brand, M. D. (2007) Mitochondrial uncouplers with an extraordinary dynamic range. *Biochem. J. 407*, 129-140.

(24) Spycher, S., Pellegrini, E., and Gasteiger, J. (2005) Use of Structure Descriptors To Discriminate between Modes of Toxic Action of Phenols. *J. Chem. Inf. Model. 45*, 200-208.