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Two novel prediction models improve predictions of skin corrosive sub-categories by test methods of OECD Test Guideline No. 431

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ABSTRACT

Alternative test methods often use prediction models (PMs) for converting endpoint measurements into predictions. Two PMs are used for the skin corrosion tests (SCTs) of the OECD Test Guideline No. 431 (TG 431). One is specific to EpiSkin™ test method, whereas EpiDerm™, SkinEthic™ RHE and epiCS® share a common PM. These methods are based on reconstructed human epidermis models wherein cell viability values are measured. Their PMs allow translating those values into sub-categories of corrosive chemicals, Category 1A (Cat1A) and a combination of Categories 1B/1C (Cat1BC), and identifying non-corrosive (NC) chemicals. EpiSkin™'s PM already results in sufficiently accurate predictions. The common PM of the three others accurately identifies all corrosive chemicals but, for sub-categorization, an important fraction of Cat1BC chemicals (40–50%) is over-predicted as Cat1A. This paper presents a post-hoc analysis of validation data on a set of n = 80 chemicals. It investigates: why this common PM causes these over-predictions and how two novel PMs that we developed (PMvar1 and PMvar2) improve the predictive capacity of these methods. PMvar1 is based on a two-step approach; PMvar2 is based on a single composite indicator of cell viability. Both showed a greater capacity to predict Cat1BC, while Cat1A correct predictions remaining at least at the same level of EpiSkin™. We suggest revising TG 431, to include the novel PMs in view of improving the predictive capacity of its SCTs.

1. Introduction

1.1. RhE test methods and their regulatory importance for the skin corrosion endpoint

Reconstructed human Epidermis (RhE) models are used in in vitro test methods for dermal acute toxicity (OECD, 2013b; OECD, 2014b). These models are constructed with human-derived epidermal keratinocytes and constitute a three dimensional structure reproducing the typical multi-layer cell organization of the epidermis.

They are used for different applications. One of them is the skin corrosion test (SCT) for the assessment of potential skin corrosive chemicals as described in the OECD Test Guideline (TG) No. 431 (OECD, 2014b). Skin corrosion is defined as irreversible damage to the skin following the application of a test chemical (UN, 2013). Clinically, necrosis is observed through the *epidermis* and into the *dermis* following the exposure to the chemical.

The assessment and classification of chemicals by the GHS United Nations (UN) Global Harmonized System of classification and labeling of chemicals (GHS) (UN, 2013) follow a tiered approach as described in its

Abbreviations: AUROC, area under ROC curve; Cat, sub-category of corrosive chemical in the UN GHS; ECHA, European Chemical Agency; OECD, Organisation for Economic Co-operation and Development; PM, prediction model; PMvar1, Prediction Model variation #1; PMvar2, Prediction Model variation #2; RhE, Reconstructed human Epidermis; ROC, Receiver Operating Characteristic; TG, Test Guideline; SCTs, skin corrosion test methods; UN GHS, United Nations Global Harmonized System of Classification and Labelling of Chemicals; v3, cell viability at 3 min; v60, cell viability at 60 min; v240, cell viability at 240 min; Cat1A, 1A category of chemicals based on the UN GHS; Cat1B, 1B category of chemicals based on the UN GHS; Cat1C, 1C category of chemicals based on the UN GHS; Cat1BC, combined categories 1B and 1C of chemicals based on the UN GHS; NC, noncorrosive category of chemicals based on the UN GHS.

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Table 1
UN GHS sub-categories of corrosive chemicals.

Category 1: corrosive (applies to authorities not using sub-categories)	Corrosive sub-categories (only applies to some authorities)	Corrosive in ≥1 of 3 animals	
		Exposure	Observation
Corrosive	1A	≤3 min	1 h
	1B	≥3 min and <1 h	14 days
	1C	≥1 and ≤4 h	14 days

Annex 3. It considers human data, animal data and in vitro data. The UN GHS labels as Category 1 chemicals that are classified as corrosive for the skin. These chemicals can be further sub-categorized (Categories 1A, 1B and 1C) depending on in vivo effects observed both during the exposure time and later during the observation period as presented in Table 1.

The identification of corrosive chemicals falls under several regulations. The UN GHS serves as a basis for the European Union System of Classification of Chemicals, implemented in the EU Classification, Labeling and Packaging Regulation (EU CLP). It is also important for transport regulation of chemicals based on the UN Transport of Dangerous Goods Regulation (TDG) (UN, 2011). The assessment of the skin corrosion potential of chemicals is also mandatory under the REACH regulation in Europe (EC, 2001; EC, 2006; EC, 2008). Chemicals labeled as corrosive will lead to taking similar protection measures, for human health, regardless of its corrosive sub-category. However sub-categorization remains essential with regard to transportation safety measures, in order to comply with the EU CLP that requires both categorization and sub-categorization (UN, 2011; UN 2013). Transport measures to be taken with regard to Cat1A are much more costly and stringent than those for Cat1B and Cat1C. Sub-categorization will also be of importance for any other (future) legislation that would be affected by the need to make these distinctions. Therefore, in vitro test methods that are sufficiently capable to make sub-categorization predictions, will be beneficial to (i) avoid unnecessary in vivo testing for sub-categorizing corrosive chemicals, (ii) reduce the unnecessary costs for the transport of Cat1B or Cat1C chemicals.

Importantly, the evaluation of skin corrosion (and irritation) using alternative methods (such as RhE) needs to be done through integrative approaches that allow for the consideration of complementary information (e.g., physicochemical properties, QSARs). An integrated approach on testing and assessment (IATA) (OECD, 2014a) is already accepted at the international level, where the RhE test methods play a key role. This IATA used the Integrated Testing Strategy (ITS) developed in the context of the REACH guidance (latest update ECHA, 2014). It is expected to lead to a revision of the "Testing and Evaluation Strategy" including the OECD TG 404 (OECD, 2002) in view of reflecting the scientific and technical progress that is presented in the IATA.

1.2. Background of the OECD TG 431: binary categorization and partial sub-categorization of corrosive chemicals with high over-prediction rates of Cat 1BC observed in 3 out of 4 methods

The initial version of the OECD TG No. 431 in 2004 allowed binary categorization of corrosive (Category 1) versus non-corrosive (NC) chemicals. This binary categorization is still presented in the current version of TG 431. Additionally this guideline was revised in 2012–2014 to allow for sub-categorization of corrosive chemicals. During this revision, the OECD expert group decided in September 2012 to consider predictions that separate Cat1A from a combination of Cat1B/Cat1C. This was done for two reasons: (i) it was deemed that the ability of RhE methods for discriminating between category 1B and category 1C was not sufficiently demonstrated and (ii) the most important differences in terms of cost/safety measures exist between Cat1A and Cat1B. In this regard, in 2013 and 2014 the updated versions of TG 431 present the sub-categorization abilities of RhE test methods by considering predictions type 'Cat1A versus Cat1BC versus non-corrosive', where "Cat1BC" represents this combination of Cat1B and Cat1C (OECD, 2013a; OECD, 2014b).

The OECD Test Guideline No. 431, in its current version, includes four skin corrosion test methods (SCTs): EpiSkin™, EpiDerm™, SkinEthic™ RHE and epiCS® (Table 2). In 2004 it included originally two validated reference methods (VRMs), based on EpiSkin™ and EpiDerm™ RhEs for the binary categorization (corrosives versus non-corrosives). It has been updated in July 2013 to include two additional test methods, SkinEthic™ RHE and epiCS® SCTs on the basis of a performance standard-based validation procedure. In that version of TG 431 it was stated that EpiDerm™, EpiSkin™ and SkinEthic™ RHE SCTs were additionally able to partially sub-categorize within the corrosive category—with distinct abilities. The guideline was then revised in July 2014 to include also epiCS® abilities to partially sub-categorize corrosive chemicals (Table 2).

TG 431 stipulates that predictions obtained with EpiSkin™ SCT can be used directly as they are, i.e., relying directly to the EpiSkin™ sub-categorization predictions (Cat1A and Cat1BC) established by its prediction model. In contrast, for EpiDerm™, SkinEthic™ RHE and epiCS®, the guideline states that chemicals for which the outcome is Cat1BC can be directly considered as Cat1BC whereas those for which the outcome is Cat1A should either be labeled as Cat1 (corrosive) or should undergo further testing to confirm the result of the sub-categorization. This latter recommendation is related to high over-prediction rates for Cat1BC (40–50% of those chemicals over-predicted as Cat1A) when using the common PM of these three test methods (OECD, 2013c; OECD, 2013a; OECD, 2014b).

1.3. Scope of the paper: possibilities for better sub-categorizing in OECD TG 431

The original common PM of EpiDerm™, SkinEthic™ RHE and epiCS® was initially developed only for the binary categorization (predictions corrosive versus non-corrosive chemicals). In the context of the revision of TG 431 described above, this common PM was adapted for sub-categorization using a cutoff of 50% cell viability value at 3 min. However it has never been systematically investigated to which extent these adaptations were appropriate for the three SCTs using this common PM and whether other PMs would provide more accurate predictions. This paper does this investigation: it examines (i) the current PMs for sub-categorization & causes of over-predictions, and (ii) proposes two new PMs for EpiDerm™, SkinEthic™ RHE and epiCS® in order to improve their predictions under the OECD TG 431. Predictions from EpiSkin™ SCT were used for comparison purposes. That means that the corresponding rates served as target values for the three other tests for which the investigation is performed (see Section 2.2).

2. Material and method

2.1. Material

The material is composed of data obtained from the four RhE methods, EpiSkin™, EpiDerm™, SkinEthic™ RHE, and epiCS® after

Table 2
Prediction types for categorization and sub-categorization of corrosive chemicals in OECD TG 431.

Test method (using OECD TG 431)	Classification of corrosive chemicals	Predictions performed
EpiSkin™, EpiDerm™, SkinEthic™ RHE, epiCS®	Categorization	Corr. vs. Non-Corr.
	(Partial) Sub-categorization	1A vs. 1BC vs. Non-Corr.

Table 3
Contingency table for evaluating the concordance of in vitro results with in vivo classification.

RhE test method considered, 80 chemicals tested in 2 or 3 independent runs, with original PM or PMvar1 or PMvar2				
In vivo	In vitro predictions			
	1A	1BC	NC	Total
1A	Correct predictions for 1A Number a → (a/n1) × 100	1A under-predicted as 1BC Number b → (b/n1) × 100	1A under-predicted as NC Number c → (c/n1) × 100	n1 = a + b + c
1BC	1BC over-predicted as 1A Number d → (d/n2) × 100	Correct predictions for 1BC Number e → (e/n2) × 100	1BC under-predicted as NC Number f → (f/n2) × 100	n2 = d + e + f
NC	NC over-predicted as 1A Number g → (g/n3) × 100	NC over-predicted as 1BC Number h → (h/n3) × 100	Correct predictions for NC Number i → (i/n3) × 100	n3 = g + h + i
Total	a + d + g	b + e + h	c + f + i	n1 + n2 + n3 = N
Overall accuracy = ((a + e + i) / N) × 100				

Table 4
Predictions models of EpiDerm™, SkinEthic™ RHE, epiCS® and EpiSkin™ used in OECD TG 431 (revised versions of 2013 and 2014).

Test method	Prediction model	Prediction
EpiDerm™SCT, SkinEthic™RHE, epiCS®	v3 < 50%	Category 1A
	{v3 ≥ 50%} AND {v60 < 15%}	Category 1BC
	{v3 ≥ 50%} AND {v60 ≥ 15%}	Non-corrosive
EpiSkin™	{v3 < 35%} AND {Any value of v60, v240 min}	Category 1A
	{v3 ≥ 35%} AND {v60 < 35%} AND {Any Value of v240} OR {v3 ≥ 35%} AND {v60 ≥ 35%} AND {v240 < 35%}	Category 1BC
	All cell viabilities ≥35%	Non-corrosive

obtaining authorization from the test methods' developers to use them. These data come from their respective validation studies (Alépée et al., 2014a; Alépée et al., 2014b; Barratt et al., 1998; Hoffmann et al., 2005; Kandárová et al., 2006; Liebsch et al., 2000; Kandárová et al., 2014 and Tornier et al., 2010), implementing their respective protocols with regard to the evaluation of skin corrosive chemicals. For the purpose of including in TG 431 the sub-categorization abilities of these four methods, additional data were generated. Results for EpiDerm™ and epiCS® have never been published and we report them here on the basis of their current PM, PMvar1 and PMvar2. Results for EpiSkin™ and SkinEthic™RHE were reported recently on the basis of their current PM only (Alépée et al., 2014a; Alépée et al., 2014b) and we report here those for SkinEthic™ on the basis of PMvar1 and PMvar2. For all methods, we performed a post-hoc analysis on these data (see Section 2.2).

For each method, the principle of the test is the same. Briefly, the test chemical is topically applied to the RhE test tissues. The output is the measurement of the optical density (OD) at different time points (3 and 60 min for all, and also 240 min for EpiSkin™) through the "MTT assay". The vital dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Thiazolyl blue tetrazolium bromide; CAS number 298-93-1] is enzymatically reduced into blue formazan that allows quantitative measurements of its OD. The OD serves to derive relative cell viability values. The value of 100% cell viability is set to the OD measured for the negative control. For each of the test chemicals, the relative cell viability is then obtained from their OD in proportion to the one of the negative control. For colored chemicals and/or for direct MTT-reducer chemicals raw OD has to be corrected prior to deriving the final cell viability. After measurements of the OD and linear conversion into cell viability values, the results provide cell viability at 3 and 60 min for EpiDerm™, SkinEthic™ RHE and epiCS®, and additionally at 240 min for EpiSkin™. These values serve to the prediction models. The set of data considers 80 chemicals (Table 9) that the four test methods tested in common. Those chemicals have been tested over 3 runs by EpiSkin™, EpiDerm™ and SkinEthic™ RHE, and over 2 runs by epiCS®. They had been previously classified using the in vivo test (OECD TG 404) and were thus included in our analysis into three 'in vivo groups' complying with OECD TG 431: in vivo non-corrosive (NC); in vivo Cat1BC (1BC); and in vivo Cat1A (1A), following what has been agreed by the OECD expert group (see Section 1.2).

2.2. Data analysis

2.2.1. Statistical analysis performed

The statistical analysis, including contingency tables, boxplots and ROC curves, was performed on Stata®12 (SE version). Boxplots show median (line within box), 25th percentile (lower hinge) and its lower adjacent value (lower adjacent line), 75th percentile (upper hinge) and its upper adjacent value (upper adjacent line), and outside values (dots).

Results from EpiSkin™ SCT were used as target values in this study for three reasons: (i) its PM was developed from the beginning for sub-categorization (Fentem et al., 1998), (ii) the OECD TG 431 states that its predictions resulting in Cat1A and Cat1BC can be accepted without need of further testing, and (iii) its over-prediction rates of Cat1BC (over-predicted as Cat1A) reaches a quite low level of 21%.

The statistical analysis aims to compare the predictions obtained with the current PMs to those that are obtained with the two novel PMs that we developed, PMvar1 and PMvar2. In order to accurately provide this 'before/after' picture, the analysis complies with the way of calculations underlying the current version of OECD TG 431 and as laid out in the annexed performance standards (OECD, 2013c; OECD, 2013a). That means that calculations of the capacity of the test methods to predict sub-categories were based on all runs generated. That implies that information generated on run level was not further processed to arrive at one single prediction per test chemical (for instance by taking the median or mean of cell viabilities or the mode of run predictions). It is acknowledged that this approach seems to increase the sample size by effectively tripling (or doubling depending on the data sets associated with the test methods) the number of data points as compared to the number of chemicals tested. This will lead to narrower Confidence Intervals. However, for the purposes of this paper, in order to be able to compare the performance of the novel prediction models with those included in the current version of TG 431, the exact same way of calculating sensitivity and specificity values had to be used, as this had been done in the analyses performed in 2012 which underlie the present version of TG 431. Therefore, the data matrices analyzed in this paper are as follows: for EpiDerm™, EpiSkin™ and SkinEthic™ RHE each of the 80

Table 5
Prediction model variation 1 (PMvar1), based on a two-step approach.

Step1: Corr. vs. Non-Corr.	{v3 < 50} OR {v3 ≥ 50 AND v60 < 15} → Corr.	v3 ≥ 50 AND v60 ≥ 15 → Non-Corr.	v3 ≥ 50 AND v60 ≥15 → Non-Corr.
Step2: Cat. 1A vs. Cat. 1B/1C	v3 < x → Cat. 1A	v3 ≥ x → Cat. 1B/1C	–

Table 6
Prediction model variation 2 (PMvar2), based on a composite cell indicator 'vfin'.

Establishment of composite indicator 'vfin'		Theoretical range of values for 'vfin'
v3 < 50	→ vfin = v3	[0; 50[
v3 ≥ 50 AND v60 <15	→ vfin = v3 + v60	[50; 115[
v3 ≥ 50 AND v60 ≥ 15	→ vfin = (2 × v3) + v60	[115; 300[

Criteria	Prediction
vfin < y	Cat1A
y ≤ vfin < z	Cat1BC
vfin ≥ z	Non-corrosive

chemicals were tested in 3 independent runs, leading to a total of 240 run predictions. For epiCS® each chemical was tested in 2 independent runs except chemical No. 58 (glycol bromoacetate (85%)) tested in 1 run only. Thus, the epiCS® data matrix consists of 159 predictions (79 chemicals tested twice plus 1 chemical tested once). The run data are presented in Table 9.

For the purpose of the analysis, 3 × 3 contingency tables such as Table 3 have been used. These tables were obtained on the basis of the original PM as included in TG 431 and the two novel PMs. This allows evaluating, for each PM used, the concordance of the in vitro sub-categorization with the in vivo sub-categorization. Results obtained in the contingency tables present the number of predictions over all runs for each method on the set of 80 chemicals. For each in vivo sub-category number and percentages of correct and possible under- and/or over-predictions are calculated (see Table 3). The overall accuracy has been also calculated. The contingency table obtained for EpiSkin™, using its current PM, serves as a reference for comparison to the other three methods for which the novel PMs are tested. Therefore for EpiDerm™, SkinEthic™ RHE and epiCS® the contingency tables were obtained according to 3 modalities, using their common current PM and the two new variations, PMvar1 and PMvar2.

Results obtained for PMvar1 and PMvar2 are compared to those obtained with the original common PM. We focus on Cat1BC where over-predictions are encountered for the three methods with the original PM. This allows assessing whether the changes of PMs resulted in significant improvements. In the end, Receiver Operating Characteristic (ROC) curves were also obtained thanks to PMvar2 and allow comparing the four methods performances for identifying each of the three sub-categories of TG 431.

For both PMvar1 and PMvar2 several cutoff values have been envisaged. For each of these possible cutoffs, we obtained 3 × 3 contingency tables with calculations of correct prediction rates, over- and under-prediction rates and overall accuracy. These tables are reported with 2 possible cutoffs according to two different goals. The first one maximizes the correct predictions for Cat1BC. The second one maximizes simultaneously correct predictions of Cat1BC and Cat1A to obtain a correct prediction value of Cat1A at least equal to EpiSkin™ SCT.

2.2.2. Original prediction models of the four RhE methods

EpiDerm™, SkinEthic™ RHE and epiCS® SCTs use the common prediction model based on cutoff values of cell viability at 3 (v3) and 60 min (v60). EpiSkin™ uses a different prediction model with cutoff values of cell viability at 3 (v3), 60 (v60) and 240 min (v240). Original prediction models included in the revised versions of 2013 and 2014 of OECD TG 431 are presented in Table 4.

In the EpiDerm™, SkinEthic™ RHE and epiCS® SCTs, the cutoff values for v3 and v60 are 50% and 15% respectively, and classification of chemicals are performed depending on the ranges of v3 and v60. In the EpiSkin™ SCT v3, v60 and v240 have all a cutoff value of 35% and combination of their possible ranges is different to classify chemicals (Table 4).

2.2.3. The two novel prediction models, PMvar1 and PMvar2

These new prediction models are applied to EpiDerm™, SkinEthic™ RHE and epiCS®. Results from EpiSkin™ are considered here as target values to be reached by EpiDerm™, SkinEthic™ RHE and epiCS® thanks to the implementation of the new prediction models, as explained in Section 2.2.

2.2.4. New prediction model variation 1 (PMvar1)

The first variation (PMvar1) consists of a two-step approach where corrosive and non-corrosive chemicals are classified based on the originally validated PM and in the second step, the group of corrosive chemicals identified undergoes sub-categorization based on different values of cell viabilities obtained at 3 min (Table 5):

Step1 Using the current prediction model to identify corrosive and non-corrosive chemicals: chemicals for which v3 ≥ 50 & v60 ≥ 15 are considered as non-corrosive, whereas all other combinations of v3 and v60 are considered as corrosive;

Step2 Chemicals identified as corrosive under step 1 are sub-categorized using a cutoff x of cell viability at 3 min (v3), i.e., predictions are Cat1A for values of v3 below x, and Cat1BC for values of v3 equal or above x.

The value of the x cutoff was determined for each of the RhEs separately by the custom-made program developed for this purpose. Boxplot graphs of the cell viabilities over the in vivo sub-categories are presented to demonstrate which cutoff is optimal for discriminating between Cat1A and Cat1BC.

2.2.5. New prediction model variation 2 (PMvar2)

PMvar2 linearly combines v3 and v60 to establish a composite cell viability indicator, noted vfin, that stands for "final cell viability", and that serves afterwards as a basis for providing predictions for sub-categorization (Table 6). Combination of v3 and v60 to obtain vfin follows different modalities (Table 6). This vfin composite indicator of cell viability aims to facilitate sub-category predictions on the basis of one value instead of considering several ranges of cell viability values

Table 7
EpiDerm™, SkinEthic™ RHE and epiCS®, obtaining of ROC curves based on PMvar2.

EpiDerm™, SkinEthic™ RHE and epiCS®	vfin composite indicator (for prediction Cat1A versus others; Non-Corr. versus others)	valt composite indicator (for predictions Cat1BC versus others)	Predicted Category
v3 < 50	vfin = v3	valt = 3 × (100 − vfin)	Cat1A
v3 ≥ 50 AND v60 < 15	vfin = v3 + v60	valt = vfin	Cat1BC
v3 ≥ 50 AND v60 ≥ 15	vfin = (2 × v3) + v60	valt = vfin	Non-corrosive

Table 8
EpiSkin™, obtaining of ROC curves derived from PMvar2.

EpiSkin™	vfin composite indicator (for predictions Cat1A versus others; or Non-Corr. versus others)	valt composite indicator (for predictions Cat1BC versus others)	Predicted category
v3 < 35	vfin = v3	valt = 4 × (100 − vfin)	Cat1A
v3 ≥ 35 AND v60 < 35	vfin = v3 + v60	valt = vfin	Cat1BC
v3 ≥ 35 AND v60 ≥ 35 AND v240 < 35	vfin = v3 + v60 + v240	valt = vfin	
v3 ≥ 35 AND v60 ≥ 35 AND v240 ≥ 35	vfin = ((4 × v3) + v60 + v240)	valt = vfin	Non-corrosive

(Table 6). Similarly as PMvar1, it ultimately aims to improve Cat1BC predictions as well as overall accuracy of the method.

This PM assumes that, theoretically, cell viability values would be included in a range of 0 to 100%. However some values of v3 are above 100% in the dataset and therefore, although PMvar2 (Table 6) would be the best possible case, some values of vfin might be still overlapping in sub-categories. Nonetheless such overlaps between sub-categories of chemicals would be reduced when compared to original prediction model. As the intervals of vfin overlap in a lower extent, discriminating between the three sub-categories may result in a greater overall accuracy.

On this theoretical basis, PMvar2 was then developed for EpiDerm™, SkinEthic™ RHE and epiCS® using the composite cell viability 'vfin', thanks to the custom-made program and the boxplot graph representing the distribution of vfin over the in vivo sub-categories. Two cutoff values were thus derived from vfin distribution, y and z respectively for discriminating Cat1A chemicals from Cat1BC and for discriminating noncorrosive chemicals from all the others. This provides in the end also a classification type Cat1A versus Cat1BC versus non-corrosive (Table 6).

2.2.6. Obtaining of ROC curves when PMvar2 is applied

Since PMvar2 is based on single cell viability composite indicator 'vfin', it allows obtaining of Receiver Operating Characteristic (ROC) curves easily. In contrast, obtaining ROC curves on the basis of several continuous variables v3, v60 (and v240) would require the use of logistic modeling (Cleves et al., 2002). For prediction types 'Cat1A versus other categories' or 'Non-Corr. versus other categories' vfin can be used. For prediction types 'Cat1BC versus other categories' the range of cell viability values of Cat1BC is between those of Cat1A and Non-Corr. and renders the obtaining of ROC curves more delicate but still feasible. For this latter case, the range of values for Cat1BC has to be different from the two others (Cat1A and Non-Corr.) and not remain in between. This is achieved by making the values of Cat1A fall under those of Non-Corr. after a linear transformation. Therefore another composite indicator 'valt' was developed on the basis of 'vfin' as outlined in Table 7.

For the case of EpiSkin™ ROC analysis also needs the development of composite cell viability indicators vfin and valt, like for the three other methods in PMvar2. However the development of these indicators has been adapted to the specific PM of EpiSkin™ (Table 8). Similarly to the other three test methods, for EpiSkin™ y and z values were chosen to implement PMvar2 to EpiSkin™ and enable the ROC analysis. Results of EpiSkin™ are presented in Table 9 with y = 40 and z = 380. ROC analysis and its results are detailed in Section 4.3.1.

3. Results

3.1. Cell viabilities obtained with the four RhE methods

Table 9 provides for each test method and each of the 80 chemicals used for the study the cell viability values at 3 and 60 min, as well the values of the composite indicator *vfin* that are used in PMvar2 (see Section 2.2). The results of in vitro sub-categorization are provided for three cases: using the current PM (as included in OECD TG 431); using PMvar1 (when taking the cutoff that gave best predictions, see Section 3.3) and using PMvar2 (when taking the cutoff that gave best predictions, see Section 3.4). According to UN GHS in vivo classifications within the set of test chemicals are: for Cat1A n = 12 chemicals; for Cat1BC n = 31 chemicals and for Non-Corr. n = 37 chemicals.

EpiDerm™ and SkinEthic™ RHE tested each of the 80 chemicals three times (runs) and provided therefore 240 predictions; epiCS® tested 79 out of 80 chemicals twice and 1 once, provided therefore 159 predictions. Cell viabilities presented are final viabilities i.e., after correction for MTT-reducing and/or colored chemicals. The composite indicator 'vfin' of cell viability as described in the Data analysis section is also presented.

3.2. Analysis of the predictive ability of the original PMs

As shown in 3 × 3 contingency tables (Tables 10–13) the original PMs in all four methods lead to high levels of correct prediction rates for Cat 1A (83–91%) and Non-Corr. chemicals (72–88%). In EpiSkin™ the over-prediction rates of Cat1BC (over-predicted as 1A) is 21.5%. In EpiDerm™, SkinEthic™ RHE, and epiCS®, that have a common PM, this over-prediction rate of Cat1BC falls within 42–54% (depending on the method).

These results constitute a verification of what TG 431 states about their sub-categorization abilities. Those are assessed in percentages of the total number of predictions:

- For EpiSkin™ correct predictions rates for Cat1A, Cat1BC, and NC are 83.33%, 76.34%, and 79.28% (Table 10).
- For the three other methods the correct prediction rates for Cat1A are close to 90% (86.11% to 91.67%), for Cat1BC slightly above or below 50% (from 46.24% to 58.06%) and for NC slightly above 70%, and therefore almost 50% of Cat1BC are over-predicted as Cat1A (Tables 11, 12 and 13).

Figs. 1, 2 and 3, present boxplot graphs for the distribution of cell viability values at 3 and 60 min in EpiDerm™, SkinEthic™ RHE and epiCS®. In all 3 figures the cutoff of 50% for v3 and 15% for v60 is represented.

For the EpiDerm™, SkinEthic™ RHE and epiCS, the cutoff of v60 = 15% seems appropriate to discriminate non-corrosive and corrosive (together 1A and 1BC) chemicals as a huge majority of non-corrosive chemicals are above this cutoff, and a majority of Cat1A and Cat1BC are below it. In contrast the cutoff of v3 = 50% does not seem optimum. For the three test methods, we observe that all Cat1A chemicals are below the cutoff, but only almost half of the Cat1BC is above it. The rest of Cat1BC are thus below this cutoff and will be over-predicted as Cat1A. This explains the high over-prediction rates observed for the three test methods regarding Cat1BC. Interestingly, all non-corrosive chemicals are above this cutoff.

For EpiSkin™, the distribution of cell viability values shows that the cutoff of 35% in its specific PM is appropriate in a majority of cases. That means that for a majority of combinations of v3, v60 and v240 (in EpiSkin™ PM) the three possible predictions (Cat1A, Cat1BC or Non-Corr.) will be discriminated. For example, the boxplot graph (Fig. 4) shows that a huge majority of Cat1A chemicals present cell viability values below 35%, and a huge majority of Non-Corr. chemicals present cell viability values over 35%. For Cat1BC, the PM requires that v3 ≥ 35 with at least either v60 or v240 < 35. The boxplot for Cat1BC chemicals

Table 9
List of tested chemicals, CASRN, in vivo classification according to UN GHS, and corresponding cell viability values and in vitro classifications performed according to several PMs. 1A: category 1A; 1BC: combined category 1B/1C; NC: non-corrosive; L: liquid; S: solid; V: viscous; green cells: correct predictions; red cells: over-predictions; blue cells: under-predictions.

EpiDerm™																						
					In vitro results Run 1						*In vitro* results Run 2						*In vitro* results Run 3					
Internal reference	Chemical name	CASRN	*In vivo*	Physical state	v3	v60	vfin	current PM	PMvar1 (x = 25)	PMvar2 (y = 25, z = 115)	v3	v60	vfin	Current PM	PMvar1 (x = 25)	PMvar2 (y = 25, z = 115)	v3	v60	vfin	current PM	PMvar1 (x = 25)	PMvar2 y = 25, z = 115)
1	o–Methoxyphenol (guaiacol)	90–05–1	NC	L	64.5	8.6	73.1	1BC	1BC	1BC	99.1	11.1	110.2	1BC	1BC	1BC	81.3	6.1	87.4	1BC	1BC	1BC
2	2,4–Xylidine (2,4–dimethylaniline)	95–68–1	NC	L	114.7	12.1	126.8	1BC	1BC	NC	73	17.9	163.9	NC	NC	NC	92	5.1	97.1	1BC	1BC	1BC
3	Phenethyl bromide (2–bromoethy benzene)	103–63–9	NC	L	112.5	71.2	296.2	NC	NC	NC	72	22.2	166.2	NC	NC	NC	98.9	97.6	295.4	NC	NC	NC
4	Butyl carbamate	592–35–8	NC	S	94.1	30.6	218.8	NC	NC	NC	84	27.8	195.8	NC	NC	NC	97.1	24	218.2	NC	NC	NC
5	L–Glutamic acid hydrochloride	138–15–8	NC	S	98.9	50.7	248.5	NC	NC	NC	92.7	78	263.4	NC	NC	NC	102.8	71.4	277	NC	NC	NC
6	1–(o–Tolyl)biguanide	93–69–6	NC	S	88.4	63.1	239.9	NC	NC	NC	84.7	79.8	249.2	NC	NC	NC	69.6	70.3	209.5	NC	NC	NC
7	Butyl glycolate (polysolvan)	7397–62–8	NC	L	83.7	19.4	186.8	NC	NC	NC	93.2	23.7	210.1	NC	NC	NC	110.4	5.9	116.3	1BC	1BC	NC
8	2–Hydroxyisobutyiric acid	594–61–6	NC	S	97.4	9.5	106.9	1BC	1BC	1BC	92	7	99	1BC	1BC	1BC	90	10.6	100.6	1BC	1BC	1BC
9	Oxalic acid dihydrate	6153–56–6	NC	S	86.6	6.3	92.9	1BC	1BC	1BC	91.6	6.4	98	1BC	1BC	1BC	100	4.2	104.2	1BC	1BC	1BC
10	Alpha–Ketoglutaric acid	328–50–7	NC	S	90.9	4.6	95.5	1BC	1BC	1BC	68.7	12	80.7	1BC	1BC	1BC	105.7	6.5	112.2	1BC	1BC	1BC
11	Sulphamic acid	5329–14–6	NC	S	93.7	6.9	100.6	1BC	1BC	1BC	81.7	7.4	89.1	1BC	1BC	1BC	85.2	8.9	94.1	1BC	1BC	1BC
12	Dodecanoic acid (lauric acid)	143–07–7	NC	S	90.7	64.4	245.8	NC	NC	NC	94.9	93.4	283.2	NC	NC	NC	104.6	84.5	293.7	NC	NC	NC
13	Sodium lauryl sulphate (20%)	151–21–3	NC	L	102.1	77.9	282.1	NC	NC	NC	101.2	108	310.4	NC	NC	NC	101.4	56.8	259.6	NC	NC	NC
14	Methyl trimethylacetate	598–98–1	NC	L	99.6	27.6	226.8	NC	NC	NC	90.4	31	211.8	NC	NC	NC	103.1	16.3	222.5	NC	NC	NC
15	4–Amino–4H–1,2,4–triazole	584–13–4	NC	S	105.7	88.2	299.6	NC	NC	NC	88.9	95.2	273	NC	NC	NC	102.8	110.8	316.4	NC	NC	NC
16	1,9–Decadiene	1647–16–1	NC	L	97.6	102.8	298	NC	NC	NC	89.2	101.9	280.3	NC	NC	NC	104.6	114.8	324	NC	NC	NC
17	Sodium carbonate (50%)	497–19–8	NC	L	104.5	46.6	255.6	NC	NC	NC	102.9	102.9	308.7	NC	NC	NC	99.6	86.7	285.9	NC	NC	NC
18	Benzylacetone (4–phenyl–2–butanone)	2550–26–7	NC	L	114.2	95.2	323.6	NC	NC	NC	89.5	131.1	310.1	NC	NC	NC	110.6	102.1	323.3	NC	NC	NC
19	Eugenol	97–53–0	NC	L	113.4	28.3	255.1	NC	NC	NC	119.1	24.5	262.7	NC	NC	NC	104.2	−10.4	93.8	1BC	1BC	1BC

20	Tetrachloroethylene	127–18–4	NC	L	109.1	47.7	265.9	NC	NC	NC	105.1	66.7	276.9	NC	NC	NC	114.8	45.5	275.1	NC	NC	NC
21	Sodium undecylenate (33%)	3398–33–2	NC	L	99.6	8.7	108.3	1BC	1BC	1BC	91.3	7.8	99.1	1BC	1BC	1BC	94.2	7	101.2	1BC	1BC	1BC
22	4–Amino–5–methoxy–2–methylbenzensulphonic acid	6471–78–9	NC	S	103.1	83	289.2	NC	NC	NC	95.9	109.2	301	NC	NC	NC	93.2	95.7	282.1	NC	NC	NC
23	Potassium hydroxide (5%)	1310–58–3	NC	L	14.3	6.9	14.3	1A	1A	1A	23	8.2	23	1A	1A	1A	15.2	7.9	15.2	1A	1A	1A
24	3,3–Dithiopropionic acid	1119–62–6	NC	S	89.2	91.2	269.6	NC	NC	NC	93.3	94.5	281.1	NC	NC	NC	88.6	94.4	271.6	NC	NC	NC
25	Isopropanol	67–63–0	NC	L	86.8	77.9	251.5	NC	NC	NC	99.6	52.1	251.3	NC	NC	NC	92.3	83.8	268.4	NC	NC	NC
26	2–Phenylalcohol (2–Phenetyl ethanol)	60–12–8	NC	L	89.4	5.4	94.8	1BC	1BC	1BC	90.9	5.9	96.8	1BC	1BC	1BC	98.7	−0.6	98.1	1BC	1BC	1BC
27	n–Butyl propionate	590–01–2	NC	L	91.4	51.1	233.9	NC	NC	NC	91.9	62.9	246.7	NC	NC	NC	90.3	48.8	229.4	NC	NC	NC
28	Methyl palmitate	112–39–0	NC	S	97.7	98.9	294.3	NC	NC	NC	85.6	84.7	255.9	NC	NC	NC	96.4	94.7	287.5	NC	NC	NC
29	Methyl laurate	111–82–0	NC	L	96.3	90.6	283.2	NC	NC	NC	97.3	104.1	298.7	NC	NC	NC	105.1	100.1	310.3	NC	NC	NC
30	Sodium bicarbonate	144–55–8	NC	S	90.8	90.2	271.8	NC	NC	NC	93.3	96.9	283.5	NC	NC	NC	102.2	94.1	298.5	NC	NC	NC
31	2–Bromobutane	78–76–2	NC	L	82.2	14.6	96.8	1BC	1BC	1BC	90.2	23.3	203.7	NC	NC	NC	98.5	19.5	216.5	NC	NC	NC
32	4–(Methylthio)–benzaldehyde	3446–89–7	NC	L	85.4	81.6	252.4	NC	NC	NC	94.8	86.3	275.9	NC	NC	NC	103.9	85.6	293.4	NC	NC	NC
33	2–Ethoxyethyl methacrylate	2370–63–0	NC	L	84.1	56.8	225	NC	NC	NC	85.7	69.1	240.5	NC	NC	NC	96.5	60.5	253.5	NC	NC	NC
34	Cinnamaldehyde	14371–10–9	NC	L	82.7	54.2	219.6	NC	NC	NC	86.6	60.6	233.8	NC	NC	NC	107.8	42.9	258.5	NC	NC	NC
35	4,4′–Methylene–bis–(2,6–ditert–butylphenol)	118–82–1	NC	S	88.7	91.3	268.7	NC	NC	NC	94.7	99.6	289	NC	NC	NC	89.5	100.6	279.6	NC	NC	NC
36	Sodium bisulfite	7631–90–5	NC	S	78	41.5	197.5	NC	NC	NC	90.2	45.1	225.5	NC	NC	NC	98.5	49.3	246.3	NC	NC	NC
37	10–Undecenoic acid	112–38–9	NC	S	86.7	51.6	225	NC	NC	NC	102.7	87	292.4	NC	NC	NC	99.7	80.1	279.5	NC	NC	NC
38	N,N–Dimethylbenzylamine	103–83–3	1BC	L	74.2	2.8	77	1BC	1BC	1BC	88.7	5.1	93.8	1BC	1BC	1BC	95.2	0.5	95.7	1BC	1BC	1BC
39	Fluoboric acid (hydrogentetrafluoroborate) (48%)	16872–11–0	1BC	L	12	6.9	12	1A	1A	1A	9	3.8	9	1A	1A	1A	9.1	7.7	9.1	1A	1A	1A
40	Maleic anhydride	108–31–6	1BC	S	45.9	0.8	45.9	1A	1BC	1BC	70.4	0.7	71.1	1BC	1BC	1BC	64.4	0.7	65.1	1BC	1BC	1BC
41	60/40 octanoic/decanoc acid	68937–75–7	1BC	L	63.2	8	71.2	1BC	1BC	1BC	67.4	5.4	72.8	1BC	1BC	1BC	67.4	8.2	75.6	1BC	1BC	1BC
42	55/45 octanoic/decanoc acid	68937–75–7	1BC	L	66.4	7.5	73.9	1BC	1BC	1BC	72.7	5.3	78	1BC	1BC	1BC	68.9	7.9	76.8	1BC	1BC	1BC
43	65/35 octanoic/decanoic acid	68937–75–7	1BC	L	75.7	7.4	83.1	1BC	1BC	1BC	76.4	6.6	83	1BC	1BC	1BC	75.1	7.6	82.7	1BC	1BC	1BC
44	N,N–Dimethylisopropylamine	996–35–0	1BC	L	62.2	11.2	73.4	1BC	1BC	1BC	70	11.4	81.4	1BC	1BC	1BC	84.9	−1.5	83.4	1BC	1BC	1BC
45	Hydrochloric acid (14.4%)	7647–01–0	1BC	L	80.8	9	89.8	1BC	1BC	1BC	65.4	8	73.4	1BC	1BC	1BC	82.8	9.2	92	1BC	1BC	1BC

(continued on next page)

Table 9 (*continued*)

46	n–Heptylamine	111–68–2	1BC	L	39.5	69.1	39.5	1A	1BC	1BC	25.9	27.2	25.9	1A	1BC	1BC	36.2	31.9	36.2	1A	1BC	1BC
47	Octanoic acid (caprylic acid)	124–07–2	1BC	L	47.5	9.1	47.5	1A	1BC	1BC	53.7	13	66.7	1BC	1BC	1BC	39.4	10.4	39.4	1A	1BC	1BC
48	Carvacrol	499–75–2	1BC	L	24	21.7	24	1A	1A	1A	68.4	−17.4	51	1BC	1BC	1BC	31.5	0.6	31.5	1A	1BC	1BC
49	2–Tert–Butylphenol	88–18–6	1BC	L	22	−4.5	22	1A	1A	1A	31.5	8.2	31.5	1A	1BC	1BC	12	2.5	12	1A	1A	1A
50	Methacrolein	78–85–3	1BC	L	77.6	5.5	83.1	1BC	1BC	1BC	40.9	20.5	40.9	1A	1BC	1BC	71.9	7	78.9	1BC	1BC	1BC
51	Lactic acid	598–82–3	1BC	L	90	3.5	93.5	1BC	1BC	1BC	72.8	7.5	80.3	1BC	1BC	1BC	99	3.9	102.9	1BC	1BC	1BC
52	Sodium bisulphate monohydrate	10034–88–5	1BC	S	80.6	9.6	90.2	1BC	1BC	1BC	79.7	14.8	94.5	1BC	1BC	1BC	93.8	7.5	101.3	1BC	1BC	1BC
53	Glyoxylic acid monohydrate	563–96–2	1BC	S	90.4	3.1	93.5	1BC	1BC	1BC	80	3.2	83.2	1BC	1BC	1BC	102.1	2	104.1	1BC	1BC	1BC
54	Sodium bisulphate	7681–38–1	1BC	S	82.6	10.1	92.7	1BC	1BC	1BC	85.6	14.3	99.9	1BC	1BC	1BC	104.5	9.7	114.2	1BC	1BC	1BC
55	Cyclohexylamine	108–91–8	1BC	L	20.6	9.3	20.6	1A	1A	1A	22.6	11.1	22.6	1A	1A	1A	17.9	7.9	17.9	1A	1A	1A
56	2–Methylbutyric acid	600–07–7	1BC	L	18.7	5	18.7	1A	1A	1A	17.2	19.6	17.2	1A	1BC	1A	9.7	4.9	9.7	1A	1A	1A
57	Glycol bromoacetate (85%)	3785–34–0	1BC	L	79.4	11.6	91	1BC	1BC	1BC	71.3	13.8	85.1	1BC	1BC	1BC	70.5	11.1	81.6	1BC	1BC	1BC
58	3–Methoxypropylamine	5332–73–0	1BC	L	6.7	6.1	6.7	1A	1A	1A	9.3	4.5	9.3	1A	1A	1A	7.7	3.8	7.7	1A	1A	1A
59	Allyl bromide	106–95–6	1BC	L	100.2	13.3	113.5	1BC	1BC	1BC	95.1	4.6	99.7	1BC	1BC	1BC	91.2	4.2	95.4	1BC	1BC	1BC
60	1–(2–Aminoethyl)piperazine	140–31–8	1BC	L	77.6	7	84.6	1BC	1BC	1BC	76.4	8.5	84.9	1BC	1BC	1BC	93.3	7.4	100.7	1BC	1BC	1BC
61	Iron(III) chloride	7705–08–0	1BC	S	37.3	13.8	37.3	1A	1BC	1BC	64.5	6.2	70.7	1BC	1BC	1BC	39.8	12.3	39.8	1A	1BC	1BC
62	Phosphoric acid	7664–38–2	1BC	L	37.5	5.2	37.5	1A	1BC	1BC	89.5	5.8	95.3	1BC	1BC	1BC	60.5	2.6	63.1	1BC	1BC	1BC
63	Propionic acid	79–09–4	1BC	L	6.2	7.7	6.2	1A	1A	1A	4.7	6	4.7	1A	1A	1A	13.5	17.3	13.5	1A	1A	1A
64	Butyric acid	107–92–6	1BC	L	6.9	3.2	6.9	1A	1A	1A	6	2.4	6	1A	1A	1A	13.2	6.7	13.2	1A	1A	1A
65	Boron trifluoride–acetic acid complex	373–61–5	1BC	L	13.1	6.3	13.1	1A	1A	1A	7.8	6.7	7.8	1A	1A	1A	3.7	6	3.7	1A	1A	1A
66	Ethanolamine	141–43–5	1BC	V	69.7	9.3	79	1BC	1BC	1BC	62.5	11	73.5	1BC	1BC	1BC	65.8	10.4	76.2	1BC	1BC	1BC
67	Hydrobromic acid (48%)	10035–10–6	1BC	L	7.6	7	7.6	1A	1A	1A	9.7	10	9.7	1A	1A	1A	3.7	9	3.7	1A	1A	1A
68	HCl + sulphuric acid + citric acid (5, 5, 5 wt%)	–	1BC	L	73.4	12.6	86	1BC	1BC	1BC	90.8	7.6	98.4	1BC	1BC	1BC	90.8	7.6	98.4	1BC	1BC	1BC
69	1,2–Diaminopropane	78–90–0	1A	L	22	14.1	22	1A	1A	1A	37	24.3	37	1A	1BC	1BC	30.2	16.2	30.2	1A	1BC	1BC
70	Phosphorus tribromide	7789–60–8	1A	L	0.6	1.2	0.6	1A	1A	1A	0.8	0.6	0.8	1A	1A	1A	6.2	3.9	6.2	1A	1A	1A
71	Boron trifluoride dihydrate	13319–75–0	1A	L	4.4	10.1	4.4	1A	1A	1A	4.2	20.5	4.2	1A	1A	1A	7	6.7	7	1A	1A	1A
72	Acrylic acid	79–10–7	1A	L	11	11.5	11	1A	1A	1A	12.1	14	12.1	1A	1A	1A	5.5	4.2	5.5	1A	1A	1A
73	Formic acid	64–18–6	1A	L	6.9	13.4	6.9	1A	1A	1A	6.5	4.5	6.5	1A	1A	1A	3.4	6.7	3.4	1A	1A	1A
74	Dichloroacetyl chloride	79–36–7	1A	L	1.3	1.4	1.3	1A	1A	1A	1.4	1.8	1.4	1A	1A	1A	0.5	0.9	0.5	1A	1A	1A
75	Silver nitrate	7761–88–8	1A	S	11.2	10.2	11.2	1A	1A	1A	19.6	23.2	19.6	1A	1A	1A	5.2	3.5	5.2	1A	1A	1A
76	Phenol	108–95–2	1A	S	22.6	13.5	22.6	1A	1A	1A	43	35	43	1A	1BC	1BC	10.3	10.3	10.3	1A	1A	1A

77	Acetic acid	64–19–7	1A	L	12.6	13.3	12.6	1A	1A	1A	15.6	22.4	15.6	1A	1A	1A	6	7.5	6	1A	1A	1A
78	Bromoacetic acid	79–08–3	1A	S	3.2	2.8	3.2	1A	1A	1A	4.2	4.2	4.2	1A	1A	1A	3.3	3.6	3.3	1A	1A	1A
79	N,N–dimethy–ldipropylenetriamine	10563–29–8	1A	L	56	1.4	57.4	1BC	1BC	1BC	52.3	11.2	63.5	1BC	1BC	1BC	95.8	5.5	101.3	1BC	1BC	1BC
80	Sulphuric acid (98%)	7664–93–9	1A	L	3.7	4.3	3.7	1A	1A	1A	3.2	3	3.2	1A	1A	1A	3.1	2.7	3.1	1A	1A	1A

SkinEthic™ RHE																						
					In vitro results Run 1						*In vitro* results Run 2						*In vitro* results Run 3					
Internal reference	Chemical name	CASRN	*In vivo*	Physical state	v3	v60	vfin	Current PM	PMvar1 (x = 18)	PMvar2 (y = 18, z = 130)	v3	v60	vfin	current PM	PMvar1 (x = 18)	PMvar2 (y = 18, z = 130)	v3	v60	vfin	current PM	PMvar1 (x = 18)	PMvar2 (y = 18, z = 130)
1	o–Methoxyphenol (guaiacol)	90–05–1	NC	L	119.3	3.6	122.9	1BC	1BC	1BC	67.1	2.2	69.3	1BC	1BC	1BC	80.6	3.2	83.8	1BC	1BC	1BC
2	2,4–Xylidine (2,4–dimethylaniline)	95–68–1	NC	L	55.4	0	55.4	1BC	1BC	1BC	45.8	4.1	45.8	1A	1BC	1BC	88.8	0	88.8	1BC	1BC	1BC
3	Phenethyl bromide (2–bromoethy benzene)	103–63–9	NC	L	103.4	83.2	290	NC	NC	NC	106.9	86.6	300.4	NC	NC	NC	123.6	61.6	308.8	NC	NC	NC
4	Butyl carbamate	592–35–8	NC	S	100.8	65.9	267.5	NC	NC	NC	86.5	4.9	91.4	1BC	1BC	1BC	103.2	6.7	109.9	1BC	1BC	1BC
5	L–Glutamic acid hydrochloride	138–15–8	NC	S	100.3	16.7	217.3	NC	NC	NC	89.1	32.8	211	NC	NC	NC	114.1	15.3	243.5	NC	NC	NC
6	1–(o–Tolyl)biguanide	93–69–6	NC	S	91.3	93.9	276.5	NC	NC	NC	80.6	82.5	243.7	NC	NC	NC	94.2	81.5	269.9	NC	NC	NC
7	Butyl glycolate (polysolvan)	7397–62–8	NC	L	111.2	26.4	248.8	NC	NC	NC	100.3	11.8	112.1	1BC	1BC	1BC	101.4	3.7	105.1	1BC	1BC	1BC
8	2–Hydroxyisobutyiricacid	594–61–6	NC	S	127.6	0.4	128	1BC	1BC	1BC	97.7	0.4	98.1	1BC	1BC	1BC	107.4	0.9	108.3	1BC	1BC	1BC
9	Oxalic acid dihydrate	6153–56–6	NC	S	112.2	0.5	112.7	1BC	1BC	1BC	86.1	0.5	86.6	1BC	1BC	1BC	103.8	0.8	104.6	1BC	1BC	1BC
10	Alpha–Ketoglutaric acid	328–50–7	NC	S	85.8	0.4	86.2	1BC	1BC	1BC	74.4	0.2	74.6	1BC	1BC	1BC	32.6	1.5	32.6	1A	1BC	1BC
11	Sulphamic acid	5329–14–6	NC	S	92.2	38.2	222.6	NC	NC	NC	96.6	96.6	289.8	NC	NC	NC	62	2.2	64.2	1BC	1BC	1BC
12	Dodecanoic acid (lauric acid)	143–07–7	NC	S	111.3	82.7	305.3	NC	NC	NC	103.6	76	283.2	NC	NC	NC	95.5	54.8	245.8	NC	NC	NC
13	Sodium lauryl sulphate (20%)	151–21–3	NC	L	99	72.3	270.3	NC	NC	NC	101.9	77	280.8	NC	NC	NC	101.7	93.5	296.9	NC	NC	NC
14	Methyl trimethylacetate	598–98–1	NC	L	97.4	37.2	232	NC	NC	NC	103.9	48.6	256.4	NC	NC	NC	92	25.1	209.1	NC	NC	NC
15	4–Amino–4H–1,2,4–triazole	584–13–4	NC	S	106	91.9	303.9	NC	NC	NC	97.5	106.6	301.6	NC	NC	NC	96.2	88.1	280.5	NC	NC	NC
16	1,9–Decadiene	1647–16–1	NC	L	101.1	73.3	275.5	NC	NC	NC	86.4	82.8	255.6	NC	NC	NC	100.6	67.3	268.5	NC	NC	NC

(continued on next page)

Table 9 (*continued*)

17	Sodium carbonate (50%)	497–19–8	NC	L	111	104.3	326.3	NC	NC	NC	95.7	82	273.4	NC	NC	NC	117.1	91.7	325.9	NC	NC	NC
18	Benzylacetone (4–phenyl–2–butanone)	2550–26–7	NC	L	86.6	67.4	240.6	NC	NC	NC	93.6	56.8	244	NC	NC	NC	94.9	65.7	255.5	NC	NC	NC
19	Eugenol	97–53–0	NC	L	60.5	5	65.5	1BC	1BC	1BC	43.5	16.2	43.5	1A	1BC	1BC	86.9	0	86.9	1BC	1BC	1BC
20	Tetrachloroethylene	127–18–4	NC	L	115.1	28.7	258.9	NC	NC	NC	87.4	37	211.8	NC	NC	NC	102.5	17.8	222.8	NC	NC	NC
21	Sodium undecylenate (33%)	3398–33–2	NC	L	98	4.3	102.3	1BC	1BC	1BC	65.5	3.9	69.4	1BC	1BC	1BC	78.3	5.7	84	1BC	1BC	1BC
22	4–Amino–5–methoxy–2–methylbenzensulphonic acid	6471–78–9	NC	S	100.8	99.4	301	NC	NC	NC	111.7	105.9	329.3	NC	NC	NC	103.6	65.5	272.7	NC	NC	NC
23	Potassium hydroxide (5%)	1310–58–3	NC	L	92.4	0	92.4	1BC	1BC	1BC	79.5	0	79.5	1BC	1BC	1BC	82.9	0	82.9	1BC	1BC	1BC
24	3,3–Dithiopropionic acid	1119–62–6	NC	S	92.1	89.2	273.4	NC	NC	NC	102.2	93.2	297.6	NC	NC	NC	102.6	99.4	304.6	NC	NC	NC
25	Isopropanol	67–63–0	NC	L	114	90.1	318.1	NC	NC	NC	82.7	75	240.4	NC	NC	NC	113.3	87.6	314.2	NC	NC	NC
26	2–Phenylalcohol (2–Phenetyl ethanol)	60–12–8	NC	L	95.4	23.6	214.4	NC	NC	NC	99.8	30.9	230.5	NC	NC	NC	105.4	2.1	107.5	1BC	1BC	1BC
27	n–Butyl propionate	590–01–2	NC	L	90.2	50.7	231.1	NC	NC	NC	73.5	36.8	183.8	NC	NC	NC	84.5	33.5	202.5	NC	NC	NC
28	Methyl palmitate	112–39–0	NC	S	119.9	115.4	355.2	NC	NC	NC	109	102.1	320.1	NC	NC	NC	108	141.3	357.3	NC	NC	NC
29	Methyl laurate	111–82–0	NC	L	92.4	84.5	269.3	NC	NC	NC	127.6	123.8	379	NC	NC	NC	90.1	102.1	282.3	NC	NC	NC
30	Sodium bicarbonate	144–55–8	NC	S	92.5	94	279	NC	NC	NC	98.1	103.6	299.8	NC	NC	NC	106.2	118.8	331.2	NC	NC	NC
31	2–Bromobutane	78–76–2	NC	L	93.3	26.4	213	NC	NC	NC	101.6	40.7	243.9	NC	NC	NC	98.7	16.7	214.1	NC	NC	NC
32	4–(Methylthio)–benzaldehyde	3446–89–7	NC	L	97.9	90	285.8	NC	NC	NC	97.8	59.3	254.9	NC	NC	NC	122.1	78	322.2	NC	NC	NC
33	2–Ethoxyethyl methacrylate	2370–63–0	NC	L	90.5	58.4	239.4	NC	NC	NC	77.4	55	209.8	NC	NC	NC	86.3	50.8	223.4	NC	NC	NC
34	Cinnamaldehyde	14371–10–9	NC	L	72.8	37.2	182.8	NC	NC	NC	86.4	15.1	187.9	NC	NC	NC	57.4	19.6	134.4	NC	NC	NC
35	4,4′–Methylene–bis–(2,6–ditert–butylphenol)	118–82–1	NC	S	104	107	315	NC	NC	NC	98.1	95.6	291.8	NC	NC	NC	111.3	89.5	312.1	NC	NC	NC
36	Sodium bislufite	7631–90–5	NC	S	112.8	68.3	293.9	NC	NC	NC	121.9	98.3	342.1	NC	NC	NC	93.1	20.4	206.6	NC	NC	NC
37	10–Undecenoic acid	112–38–9	NC	S	62.1	54.8	179	NC	NC	NC	111.5	81.4	304.4	NC	NC	NC	80.6	16.7	177.9	NC	NC	NC
38	N,N–Dimethylbenzylamine	103–83–3	1BC	L	63.5	0.2	63.7	1BC	1BC	1BC	62.1	8.4	70.5	1BC	1BC	1BC	40.6	10.1	40.6	1A	1BC	1BC
39	Fluoboric acid (hydrogentetrafluoroborate) (48%)	16872–11–0	1BC	L	25.6	0.6	25.6	1A	1BC	1BC	26.7	0.3	26.7	1A	1BC	1BC	25.5	0.1	25.5	1A	1BC	1BC
40	Maleic anhydride	108–31–6	1BC	S	62.2	0.2	62.4	1BC	1BC	1BC	40.2	0.1	40.2	1A	1BC	1BC	51.5	0.2	51.7	1BC	1BC	1BC
41	60/40 octanoic/decanoc acid	68937–75–7	1BC	L	53.2	5.9	59.1	1BC	1BC	1BC	55.6	3.3	58.9	1BC	1BC	1BC	78	7.6	85.6	1BC	1BC	1BC
42	55/45 octanoic/decanoc acid	68937–75–7	1BC	L	64.5	8.3	72.8	1BC	1BC	1BC	57	4	61	1BC	1BC	1BC	61.2	3.3	64.5	1BC	1BC	1BC

43	65/35 octanoic/decanoic acid	68937–75–7	1BC	L	51.8	5.7	57.5	1BC	1BC	1BC	59.3	2.6	61.9	1BC	1BC	1BC	60.9	4.3	65.2	1BC	1BC	1BC
44	N,N–dimethylisopropylamine	996–35–0	1BC	L	3.9	4.1	3.9	1A	1A	1A	44.8	3.1	44.8	1A	1BC	1BC	19.8	3.1	19.8	1A	1BC	1BC
45	Hydrochloric acid (14.4%)	7647–01–0	1BC	L	97.2	1.5	98.7	1BC	1BC	1BC	88	0.5	88.5	1BC	1BC	1BC	90.6	3.8	94.4	1BC	1BC	1BC
46	n–Heptylamine	111–68–2	1BC	L	0	0	0	1A	1A	1A	0	0	0	1A	1A	1A	0	0	0	1A	1A	1A
47	Octanoic acid (caprylic acid)	124–07–2	1BC	L	27.8	2.3	27.8	1A	1BC	1BC	63.9	2.4	66.3	1BC	1BC	1BC	19.1	1.7	19.1	1A	1BC	1BC
48	Carvacrol	499–75–2	1BC	L	15.7	9.8	15.7	1A	1A	1A	12.9	0.3	12.9	1A	1A	1A	31	4.6	31	1A	1BC	1BC
49	2–tert–Butylphenol	88–18–6	1BC	L	8.6	0	8.6	1A	1A	1A	2.4	3.3	2.4	1A	1A	1A	2.3	0	2.3	1A	1A	1A
50	Methacrolein	78–85–3	1BC	L	59.6	5.2	64.8	1BC	1BC	1BC	54.1	6.2	60.3	1BC	1BC	1BC	63.3	10.7	74	1BC	1BC	1BC
51	Lactic acid	598–82–3	1BC	L	76.3	1.9	78.2	1BC	1BC	1BC	76.7	0.4	77.1	1BC	1BC	1BC	91.2	0.4	91.6	1BC	1BC	1BC
52	Sodium bisulphate monohydrate	10034–88–5	1BC	S	121.3	4.2	125.5	1BC	1BC	1BC	109.7	9.9	119.6	1BC	1BC	1BC	110.4	84.5	305.3	NC	NC	NC
53	Glyoxylic acid monohydrate	563–96–2	1BC	S	82.1	0.3	82.4	1BC	1BC	1BC	94.8	0.2	95	1BC	1BC	1BC	100.3	0.3	100.6	1BC	1BC	1BC
54	Sodium bisulphate	7681–38–1	1BC	S	123.8	4.9	128.7	1BC	1BC	1BC	105.8	10.6	116.4	1BC	1BC	1BC	92.1	6.2	98.3	1BC	1BC	1BC
55	Cyclohexylamine	108–91–8	1BC	L	4.4	2.4	4.4	1A	1A	1A	9.5	1.4	9.5	1A	1A	1A	6.7	3	6.7	1A	1A	1A
56	2–Methylbutyric acid	600–07–7	1BC	L	3.1	1.1	3.1	1A	1A	1A	6.1	0	6.1	1A	1A	1A	18.6	2.1	18.6	1A	1BC	1BC
57	Glycol bromoacetate (85%)	3785–34–0	1BC	L	70.1	0.6	70.7	1BC	1BC	1BC	62.6	0.6	63.2	1BC	1BC	1BC	59.3	0.4	59.7	1BC	1BC	1BC
58	3–Methoxypropylamine	5332–73–0	1BC	L	13.9	20.1	13.9	1A	1A	1A	16.6	1.7	16.6	1A	1A	1A	9.2	0	9.2	1A	1A	1A
59	Allyl bromide	106–95–6	1BC	L	48.9	1.6	48.9	1A	1BC	1BC	45.8	1.4	45.8	1A	1BC	1BC	45	2.3	45	1A	1BC	1BC
60	1–(2–Aminoethyl)piperazine	140–31–8	1BC	L	97	48.3	242.3	NC	NC	NC	95.3	66.5	257.1	NC	NC	NC	86.5	58.6	231.6	NC	NC	NC
61	Iron(III) chloride	7705–08–0	1BC	S	102.6	43.4	248.6	NC	NC	NC	89.8	101.1	280.7	NC	NC	NC	93.5	83.7	270.7	NC	NC	NC
62	Phosphoric acid	7664–38–2	1BC	L	84.1	1.8	85.9	1BC	1BC	1BC	82	1	83	1BC	1BC	1BC	119.8	0.3	120.1	1BC	1BC	1BC
63	Propionic acid	79–09–4	1BC	L	1.7	4.8	1.7	1A	1A	1A	4.1	3.9	4.1	1A	1A	1A	2.2	2.6	2.2	1A	1A	1A
64	Butyric acid	107–92–6	1BC	L	4.1	3.3	4.1	1A	1A	1A	4.7	1.6	4.7	1A	1A	1A	1.8	0.8	1.8	1A	1A	1A
65	Boron trifluoride–acetic acid complex	373–61–5	1BC	L	5.5	3.1	5.5	1A	1A	1A	1.1	2.5	1.1	1A	1A	1A	0.2	0.9	0.2	1A	1A	1A
66	Ethanolamine	141–43–5	1BC	V	73.8	0.7	74.5	1BC	1BC	1BC	86.5	5.6	92.1	1BC	1BC	1BC	59.2	0	59.2	1BC	1BC	1BC
67	Hydrobromic acid (48%)	10035–10–6	1BC	L	4.7	3.6	4.7	1A	1A	1A	0.1	1.8	0.1	1A	1A	1A	2.9	0.8	2.9	1A	1A	1A
68	HCl + sulphuric acid + citric acid (5, 5, 5 wt.%)	–	1BC	L	96.3	2.8	99.1	1BC	1BC	1BC	104	2	106	1BC	1BC	1BC	100.4	2.8	103.2	1BC	1BC	1BC
69	1,2–Diaminopropane	78–90–0	1A	L	63.3	0	63.3	1BC	1BC	1BC	69.1	6.2	75.3	1BC	1BC	1BC	17.5	6.6	17.5	1A	1A	1A
70	Phosphorus tribromide	7789–60–8	1A	L	0.7	1.7	0.7	1A	1A	1A	0.9	0.8	0.9	1A	1A	1A	0.4	0.4	0.4	1A	1A	1A
71	Boron trifluoride dihydrate	13319–75–0	1A	L	0.1	0	0.1	1A	1A	1A	0.1	0	0.1	1A	1A	1A	1.5	3.7	1.5	1A	1A	1A

(continued on next page)

Table 9 (*continued*)

72	Acrylic acid	79–10–7	1A	L	2.3	1.6	2.3	1A	1A	1A	0.6	1.1	0.6	1A	1A	1A	2.7	1.9	2.7	1A	1A	1A
73	Formic acid	64–18–6	1A	L	1.9	2.8	1.9	1A	1A	1A	1.2	3.2	1.2	1A	1A	1A	2.3	3.5	2.3	1A	1A	1A
74	Dichloroacetyl chloride	79–36–7	1A	L	0.3	0.8	0.3	1A	1A	1A	0.4	2.3	0.4	1A	1A	1A	0.1	0.1	0.1	1A	1A	1A
75	Silver nitrate	7761–88–8	1A	S	3.1	9.5	3.1	1A	1A	1A	31.7	11.2	31.7	1A	1BC	1BC	5	15.3	5	1A	1A	1A
76	Phenol	108–95–2	1A	S	10.2	6.2	10.2	1A	1A	1A	6	5	6	1A	1A	1A	6.5	4.3	6.5	1A	1A	1A
77	Acetic acid	64–19–7	1A	L	2.2	2.7	2.2	1A	1A	1A	2.3	0.8	2.3	1A	1A	1A	1.1	2	1.1	1A	1A	1A
78	Bromoacetic acid	79–08–3	1A	S	1	2.6	1	1A	1A	1A	2.2	2.3	2.2	1A	1A	1A	2.2	2.9	2.2	1A	1A	1A
79	N,N–Dimethyl–dipropylenetriamine	10563–29–8	1A	L	100.5	7.6	108.1	1BC	1BC	1BC	83.6	5.7	89.3	1BC	1BC	1BC	91.7	4.3	96	1BC	1BC	1BC
80	Sulphuric acid (98%)	7664-93-9	1A	L	1.4	1.4	1.4	1A	1A	1A	1.2	1.3	1.2	1A	1A	1A	2.4	0.7	2.4	1A	1A	1A

epiCS®																	
					In vitro results Run 1						*In vitro* results Run 2						
Internal reference	Chemical name	CASRN	*In vivo*	Physical state	v3	v60	vfin	Current PM	PMvar1 (x = 15)	PMvar2 (y = 18, z = 130)	v3	v60	vfin	Current PM	PMvar1 (x = 15)	PMvar2 (y = 18, z = 130)	
1	o–Methoxyphenol (guaiacol)	90–05–1	NC	L	77.75	9.09	86.84	1BC	1BC	1BC	98.31	1.79	100.1	1BC	1BC	1BC	
2	2,4–Xylidine (2,4–dimethylaniline)	95–68–1	NC	L	91.2	22.44	204.84	NC	NC	NC	84.1	2.8	86.9	1BC	1BC	1BC	
3	Phenethyl bromide (2–bromoethy benzene)	103–63–9	NC	L	93.97	55.26	243.2	NC	NC	NC	80.87	58.81	220.55	NC	NC	NC	
4	Butyl carbamate	592–35–8	NC	S	116.29	64.6	297.18	NC	NC	NC	150.7	46.2	347.6	NC	NC	NC	
5	L–Glutamic acid hydrochloride	138–15–8	NC	S	130.3	126.5	387.1	NC	NC	NC	151.5	144.4	447.4	NC	NC	NC	
6	1–(o–Tolyl)biguanide	93–69–6	NC	S	101.87	98	301.74	NC	NC	NC	97.57	76.28	271.42	NC	NC	NC	
7	Butyl glycolate (polysolvan)	7397–62–8	NC	L	101.49	8.6	110.09	1BC	1BC	1BC	105	8.84	113.84	1BC	1BC	1BC	
8	2–Hydroxyisobutyiric acid	594–61–6	NC	S	64.11	2.48	66.59	1BC	1BC	1BC	57.83	2.85	60.68	1BC	1BC	1BC	
9	Oxalic acid dihydrate	6153–56–6	NC	S	108.2	8.2	116.4	1BC	1BC	1BC	116.6	3.8	120.4	1BC	1BC	1BC	
10	alpha–Ketoglutaric acid	328–50–7	NC	S	94.4	3.3	97.7	1BC	1BC	1BC	106.1	3.8	109.9	1BC	1BC	1BC	
11	Sulphamic acid	5329–14–6	NC	S	104	2.9	106.9	1BC	1BC	1BC	105.1	3.8	108.9	1BC	1BC	1BC	
12	Dodecanoic acid (lauric acid)	143–07–7	NC	S	104.29	93.05	301.63	NC	NC	NC	94.34	76	264.68	NC	NC	NC	
13	Sodium lauryl sulphate (20%)	151–21–3	NC	L	84.49	62.56	231.54	NC	NC	NC	114.69	51.64	281.02	NC	NC	NC	
14	Methyl trimethylacetate	598–98–1	NC	L	93	22.69	208.69	NC	NC	NC	113.61	18.64	245.86	NC	NC	NC	

15	4–Amino–4H–1,2,4–triazole	584–13–4	NC	S	98	81	277	NC	NC	NC	106.9	94	307.8	NC	NC	NC
16	1,9–Decadiene	1647–16–1	NC	L	87.03	16.57	190.63	NC	NC	NC	104.82	82.06	291.7	NC	NC	NC
17	Sodium carbonate (50%)	497–19–8	NC	L	89.4	98.6	277.4	NC	NC	NC	90.9	52.3	234.1	NC	NC	NC
18	Benzylacetone (4–phenyl–2–butanone)	2550–26–7	NC	L	76.57	44.87	198.01	NC	NC	NC	83.68	55.74	223.1	NC	NC	NC
19	Eugenol	97–53–0	NC	L	92.65	28.63	213.93	NC	NC	NC	63.05	20.61	146.71	NC	NC	NC
20	Tetrachloroethylene	127–18–4	NC	L	87.22	27.5	201.94	NC	NC	NC	88.2	19.6	196	NC	NC	NC
21	Sodium undecylenate (33%)	3398–33–2	NC	L	69.81	7.13	76.93999	1BC	1BC	1BC	72.68	8.56	81.24	1BC	1BC	1BC
22	4–Amino–5–methoxy–2–methylbenzensulphonic acid	6471–78–9	NC	S	100.57	111.03	312.17	NC	NC	NC	106.27	113.09	325.63	NC	NC	NC
23	Potassium hydroxide (5%)	1310–58–3	NC	L	91.23	−1.23	90	1BC	1BC	1BC	62.26	6.66	68.92	1BC	1BC	1BC
24	3,3–Dithiopropionic acid	1119–62–6	NC	S	91.01	108.71	290.73	NC	NC	NC	109.88	120.34	340.1	NC	NC	NC
25	Isopropanol	67–63–0	NC	L	103.83	119.94	327.6	NC	NC	NC	98.76	106.08	303.6	NC	NC	NC
26	2–Phenylalcohol (2–Phenetyl etanol)	60–12–8	NC	L	102.3	1.66	103.96	1BC	1BC	1BC	99.19	1.89	101.08	1BC	1BC	1BC
27	n–Butyl propionate	590–01–2	NC	L	100.43	19.46	220.32	NC	NC	NC	70.77	17.72	159.26	NC	NC	NC
28	Methyl palmitate	112–39–0	NC	S	93.91	100.11	287.93	NC	NC	NC	102.39	112.48	317.26	NC	NC	NC
29	Methyl laurate	111–82–0	NC	L	106.58	98.38	311.54	NC	NC	NC	94.1	98.25	286.45	NC	NC	NC
30	Sodium bicarbonate	144–55–8	NC	S	97.8	103	298.6	NC	NC	NC	128.8	129.9	387.5	NC	NC	NC
31	2–Bromobutane	78–76–2	NC	L	78.03	21.88	177.94	NC	NC	NC	74.61	16.97	166.19	NC	NC	NC
32	4–(Methylthio)–benzaldehyde	3446–89–7	NC	L	89.34	62.32	241	NC	NC	NC	83.89	71.52	239.3	NC	NC	NC
33	2–Ethoxyethyl methacrylate	2370–63–0	NC	L	94.97	59.74	249.68	NC	NC	NC	86.08	36.04	208.2	NC	NC	NC
34	Cinnamaldehyde	14371–10–9	NC	L	76.73	13.31	90.04	1BC	1BC	1BC	91.1	4.47	95.57	1BC	1BC	1BC
35	4,4'–Methylene–bis–(2,6–ditert–butylphenol)	118–82–1	NC	S	93.06	87.95	274.07	NC	NC	NC	101.73	112.69	316.15	NC	NC	NC
36	Sodium bislufite	7631–90–5	NC	S	103.76	91.51	299.03	NC	NC	NC	86.49	77.25	250.23	NC	NC	NC
37	10–Undecenoic acid	112–38–9	NC	S	69.33	34.47	173.13	NC	NC	NC	63.88	15.86	143.62	NC	NC	NC
38	N,N–Dimethylbenzylamine	103–83–3	1BC	L	27.35	1.3	27.35	1A	1BC	1BC	31.82	6.07	31.82	1A	1BC	1BC
39	Fluoboric acid (hydrogentetrafluoroborate) (48%)	16872–11–0	1BC	L	1.99	1.96	1.99	1A	1A	1A	2.97	2.68	2.97	1A	1A	1A
40	Maleic anhydride	108–31–6	1BC	S	26.12	3.8	26.12	1A	1BC	1BC	85.62	2.64	88.26	1BC	1BC	1BC
41	60/40 Octanoic/decanoc acid	68937–75–7	1BC	L	50.48	5.36	55.84	1BC	1BC	1BC	51.03	5.58	56.61	1BC	1BC	1BC
42	55/45 Octanoic/decanoc acid	68937–75–7	1BC	L	57.09	6.29	63.38	1BC	1BC	1BC	68.36	5.65	74.01	1BC	1BC	1BC
43	65/35 Octanoic/decanoic acid	68937–75–7	1BC	L	51.91	4.42	56.33	1BC	1BC	1BC	52.34	4.8	57.14	1BC	1BC	1BC
44	N,N–Dimethylisopropylamine	996–35–0	1BC	L	61.86	−1.41	60.45	1BC	1BC	1BC	86.71	10.69	97.4	1BC	1BC	1BC
45	Hydrochloric acid (14.4%)	7647–01–0	1BC	L	84.13	2.93	87.06	1BC	1BC	1BC	58.58	5.9	64.48	1BC	1BC	1BC

(continued on next page)

Table 9 (*continued*)

46	n–Heptylamine	111–68–2	1BC	L	−13.73	8.31	−13.73	1A	1A	1A	23.2	6.46	23.2	1A	1BC	1BC
47	Octanoic acid (caprylic acid)	124–07–2	1BC	L	27.32	3.03	27.32	1A	1BC	1BC	23.2	2.9	23.2	1A	1BC	1BC
48	Carvacrol	499–75–2	1BC	L	90.67	3.65	94.32	1BC	1BC	1BC	12.83	2.44	12.83	1A	1A	1A
49	2–tert–Butylphenol	88–18–6	1BC	L	10.26	4.95	10.26	1A	1A	1A	7.36	−1.71	7.36	1A	1A	1A
50	Methacrolein	78–85–3	1BC	L	58.2	0.9	59.1	1BC	1BC	1BC	66	3.8	69.8	1BC	1BC	1BC
51	Lactic acid	598–82–3	1BC	L	74.81	0.01	74.82	1BC	1BC	1BC	86.05	2.24	88.29	1BC	1BC	1BC
52	Sodium bisulphate monohydrate	10034–88–5	1BC	S	117.13	14.3	131.43	1BC	1BC	NC	105.97	4.28	110.25	1BC	1BC	1BC
53	Glyoxylic acid monohydrate	563–96–2	1BC	S	100.82	2.87	103.69	1BC	1BC	1BC	88.82	2.68	91.5	1BC	1BC	1BC
54	Sodium bisulphate	7681–38–1	1BC	S	86.28	84.64	257.2	NC	NC	NC	90.54	97.1	278.18	NC	NC	NC
55	Cyclohexylamine	108–91–8	1BC	L	0.61	0.1	0.61	1A	1A	1A	0.53	1.31	0.53	1A	1A	1A
56	2–Methylbutyric acid	600–07–7	1BC	L	7.39	2.39	7.39	1A	1A	1A	4.17	2.44	4.17	1A	1A	1A
57	Glycol bromoacetate (85%)	3785–34–0	1BC	L	53.82	0.25	54.07	1BC	1BC	1BC						
58	3–Methoxypropylamine	5332–73–0	1BC	L	10.87	0.67	10.87	1A	1A	1A	6.62	4.76	6.62	1A	1A	1A
59	Allyl bromide	106–95–6	1BC	L	83.08	13.14	96.22	1BC	1BC	1BC	57.87	3.48	61.35	1BC	1BC	1BC
60	1–(2–Aminoethyl)piperazine	140–31–8	1BC	L	102.09	13.47	115.56	1BC	1BC	1BC	77.87	3.44	81.31	1BC	1BC	1BC
61	Iron(III) chloride	7705–08–0	1BC	S	106.13	103.66	315.92	NC	NC	NC	83.27	80.26	246.8	NC	NC	NC
62	Phosphoric acid	7664–38–2	1BC	L	35.77	3.7	35.77	1A	1BC	1BC	7.78	2.48	7.78	1A	1A	1A
63	Propionic acid	79–09–4	1BC	L	2.94	3.22	2.94	1A	1A	1A	2.26	4.42	2.26	1A	1A	1A
64	Butyric acid	107–92–6	1BC	L	0.6	0.18	0.6	1A	1A	1A	3.21	2.26	3.21	1A	1A	1A
65	Boron trifluoride–acetic acid complex	373–61–5	1BC	L	1.8	3.96	1.8	1A	1A	1A	23.51	3.9	23.51	1A	1BC	1BC
66	Ethanolamine	141–43–5	1BC	V	106.73	3.12	109.85	1BC	1BC	1BC	71.68	2.57	74.25	1BC	1BC	1BC
67	Hydrobromic acid (48%)	10035–10–6	1BC	L	2.15	5.84	2.15	1A	1A	1A	1.95	5.09	1.95	1A	1A	1A
68	HCl + sulphuric acid + citric acid (5, 5, 5 wt.%)	–	1BC	L	97.54	2.99	100.53	1BC	1BC	1BC	102.93	4.97	107.9	1BC	1BC	1BC
69	1,2–Diaminopropane	78–90–0	1A	L	2.89	4.33	2.89	1A	1A	1A	5.2	3.34	5.2	1A	1A	1A
70	Phosphorus tribromide	7789–60–8	1A	L	14.91	1.02	14.91	1A	1A	1A	2.37	5.83	2.37	1A	1A	1A
71	Boron trifluoride dihydrate	13319–75–0	1A	L	6.07	2.58	6.07	1A	1A	1A	12.02	3.66	12.02	1A	1A	1A
72	Acrylic acid	79–10–7	1A	L	4.93	3.74	4.93	1A	1A	1A	3.56	2.45	3.56	1A	1A	1A
73	Formic acid	64–18–6	1A	L	1.9	2.9	1.9	1A	1A	1A	1.74	3.36	1.74	1A	1A	1A
74	Dichloroacetyl chloride	79–36–7	1A	L	1.59	1.65	1.59	1A	1A	1A	1.43	1.46	1.43	1A	1A	1A
75	Silver nitrate	7761–88–8	1A	S	17.5	20.8	17.5	1A	1BC	1A	0.42	9.33	0.42	1A	1A	1A
76	Phenol	108–95–2	1A	S	9.7	5.3	9.7	1A	1A	1A	9.66	4.84	9.66	1A	1A	1A

77	Acetic acid	64–19–7	1A	L	3.02	4.31	3.02	1A	1A	1A		2.35	4.21	2.35	1A	1A	1A			
78	Bromoacetic acid	79–08–3	1A	S	4.4	3.7	4.4	1A	1A	1A		2.28	3.42	2.28	1A	1A	1A			
79	N,N–Dimethyl–dipropylenetriamine	10563–29–8	1A	L	69.68	−1.01	68.67	1BC	1BC	1BC		85.7	6.62	92.32	1BC	1BC	1BC			
80	Sulphuric acid (98%)	7664–93–9	1A	L	2.8	2.33	2.8	1A	1A	1A		2.71	2.23	2.71	1A	1A	1A			

EpiSkin™–results of EpiSkin™ are presented for the purpose of the ROC analysis performed on the basis of PMvar2																							
					In vitro results Run 1						*In vitro* results Run 2						*In vitro* results Run 3					
Internal reference	Chemical name	CASRN	*In vivo*	Physical state	v3	v60	V240	vfin	Current PM	PMvar2 (y = 40, z = 380)	v3	v60	v240	vfin	Current PM	PMvar2 (y = 40, z = 380)	v3	v60	v240	vfin	Current PM	PMvar2 (y = 18, z = 130)
1	o–Methoxyphenol (guaiacol)	90–05–1	NC	L	109.5	64.8	36.1	538.9	NC	NC	98.6	49.9	37.3	481.6	NC	NC	83.7	35.5	15.7	134.9	1BC	1BC
2	2,4–Xylidine (2,4–dimethylaniline)	95–68–1	NC	L	109.8	101.2	31.2	242.2	1BC	1BC	124.5	98.3	77.7	674	NC	NC	139.5	93.8	50.8	702.6	NC	NC
3	Phenethyl bromide (2–bromoethy benzene)	103–63–9	NC	L	141	127.2	117.2	808.4	NC	NC	144.7	137.5	139.4	855.7	NC	NC	147.6	173	182.7	946.1	NC	NC
4	Butyl carbamate	592–35–8	NC	S	110.9	113.9	91.1	648.6	NC	NC	108.5	112.1	86.2	632.3	NC	NC	91.6	107.6	93.5	567.5	NC	NC
5	L–Glutamic acid hydrochloride	138–15–8	NC	S	106.7	84.2	33.6	224.5	1BC	1BC	95.3	90.2	68.6	540	NC	NC	94.5	94	42.8	514.8	NC	NC
6	1–(o–Tolyl)biguanide	93–69–6	NC	S	115.6	110.3	104.9	677.6	NC	NC	94.6	98.5	98.9	575.8	NC	NC	94.6	107.1	104	589.5	NC	NC
7	Butyl glycolate (polysolvan)	7397–62–8	NC	L	110.9	108.2	82.6	634.4	NC	NC	88.7	92.6	74.5	521.9	NC	NC	96.1	105.7	94.1	584.2	NC	NC
8	2–Hydroxyisobutyiric acid	594–61–6	NC	S	94.7	42.6	5.4	142.7	1BC	1BC	95.5	41.4	3.7	140.6	1BC	1BC	103	35	6.7	144.7	1BC	1BC
9	Oxalic acid dihydrate	6153–56–6	NC	S	103.6	38.9	4.2	146.7	1BC	1BC	91.5	51.9	17.5	160.9	1BC	1BC	109.6	50.3	6.4	166.3	1BC	1BC
10	alpha–Ketoglutaric acid	328–50–7	NC	S	71.5	18.1	4.1	89.6	1BC	1BC	101.7	19.8	6.6	121.5	1BC	1BC	101.7	10.5	5.7	112.2	1BC	1BC
11	Sulphamic acid	5329–14–6	NC	S	102.9	26.6	20.9	129.5	1BC	1BC	97.9	19.4	0.6	117.3	1BC	1BC	111.2	34.2	2.5	145.4	1BC	1BC
12	Dodecanoic acid (lauric acid)	143–07–7	NC	S	102	117.4	120.8	646.2	NC	NC	104	111.5	141.7	669.2	NC	NC	104.6	94.4	108.4	621.2	NC	NC
13	Sodium lauryl sulphate (20%)	151–21–3	NC	L	104.9	100.1	79	598.7	NC	NC	114	114.6	94.2	664.8	NC	NC	89	90.2	80.6	526.8	NC	NC
14	Methyl trimethylacetate	598–98–1	NC	L	111.3	117.4	75.3	637.9	NC	NC	106.5	100.9	94	620.9	NC	NC	116.2	109.3	97.2	671.3	NC	NC
15	4–Amino–4H–1,2,4–triazole	584–13–4	NC	S	116.8	120.6	79.6	667.4	NC	NC	105.9	106	99.4	629	NC	NC	105	105.6	97.2	622.8	NC	NC
16	1,9–Decadiene	1647–16–1	NC	L	109.5	112.4	87.8	638.2	NC	NC	114.3	136.3	126.7	720.2	NC	NC	137.6	128.6	110.2	789.2	NC	NC
17	Sodium carbonate (50%)	497–19–8	NC	L	103.8	65.2	60.7	541.1	NC	NC	140	107.5	44.7	712.2	NC	NC	73.8	79	43.5	417.7	NC	NC

(continued on next page)

Table 9 (*continued*)

18	Benzylacetone (4–phenyl–2–butanone)	2550–26–7	NC	L	133.2	141.2	137.1	811.1	NC	NC	134.7	151.3	149.9	840	NC	NC	150.3	142.2	143.4	886.8	NC	NC
19	Eugenol	97–53–0	NC	L	188.4	122.3	42.7	918.6	NC	NC	121.7	86	12.1	219.8	1BC	1BC	132.9	74.8	14.2	221.9	1BC	1BC
20	Tetrachloroethylene	127–18–4	NC	L	106.5	88.4	68.5	582.9	NC	NC	113.1	116.3	56.2	624.9	NC	NC	112	97.4	75	620.4	NC	NC
21	Sodium undecylenate (33%)	3398–33–2	NC	L	137.6	36	10.7	184.3	1BC	1BC	127.7	40.2	11	178.9	1BC	1BC	149.7	55	16.5	221.2	1BC	1BC
22	4–Amino–5–methoxy–2–methylbenzensulphonic acid	6471–78–9	NC	S	99.9	115.6	98.6	613.8	NC	NC	105.7	92.3	85.1	600.2	NC	NC	98.2	112.6	101.2	606.6	NC	NC
23	Potassium hydroxide (5%)	1310–58–3	NC	L	72.3	24.6	16.5	96.9	1BC	1BC	68.5	30.1	14.6	98.6	1BC	1BC	94.8	18.9	26.8	113.7	1BC	1BC
24	3,3–Dithiopropionic acid	1119–62–6	NC	S	113.4	117.5	105.8	676.9	NC	NC	107.9	108.9	95.7	636.2	NC	NC	110.3	102.1	113.1	656.4	NC	NC
25	Isopropanol	67–63–0	NC	L	98.9	84.4	88.5	568.5	NC	NC	91.8	87.8	80.8	535.8	NC	NC	98.1	100.2	94.7	587.3	NC	NC
26	2–Phenylalcohol (2–Phenetyl etanol)	60–12–8	NC	L	102.1	98.1	91.5	598	NC	NC	110.5	108.3	126.1	676.4	NC	NC	88.1	87.6	100.1	540.1	NC	NC
27	n–Butyl propionate	590–01–2	NC	L	106.3	80.5	58.7	564.4	NC	NC	111.5	105.7	63.1	614.8	NC	NC	95.3	70.4	47.9	499.5	NC	NC
28	Methyl palmitate	112–39–0	NC	S	108.3	105.3	97.2	635.7	NC	NC	115.7	109.3	92.1	664.2	NC	NC	96.5	80.1	103.1	569.2	NC	NC
29	Methyl laurate	111–82–0	NC	L	100.9	100.2	93.6	597.4	NC	NC	102.4	95.8	100.5	605.9	NC	NC	105.8	117.9	110.8	651.9	NC	NC
30	Sodium bicarbonate	144–55–8	NC	S	94.3	95.1	90.7	563	NC	NC	105	102.1	115.3	637.4	NC	NC	102.8	92.3	97.4	600.9	NC	NC
31	2–Bromobutane	78–76–2	NC	L	105.6	85.5	35.3	543.2	NC	NC	101.8	95.1	103.2	605.5	NC	NC	133.8	104	54.3	693.5	NC	NC
32	4–(Methylthio)–benzaldehyde	3446–89–7	NC	L	136.7	150.4	138.1	835.3	NC	NC	143.7	150.3	150.7	875.8	NC	NC	142.2	158.3	154.2	881.3	NC	NC
33	2–Ethoxyethyl methacrylate	2370–63–0	NC	L	132	133.2	125.8	787	NC	NC	142	139.6	164.5	872.1	NC	NC	133.1	139.7	154.6	826.7	NC	NC
34	Cinnamaldehyde	14371–10–9	NC	L	142.1	125.1	99.3	792.8	NC	NC	134.5	97.3	80	715.3	NC	NC	138.5	94.2	48.8	697	NC	NC
35	4,4´–Methylene–bis–(2,6–ditert–butylphenol)	118–82–1	NC	S	109.5	100.9	102.7	641.6	NC	NC	110.3	104.8	100.7	646.7	NC	NC	110.6	100.5	95.4	638.3	NC	NC
36	Sodium bislufite	7631–90–5	NC	S	94.9	67.6	42.3	489.5	NC	NC	89.4	92.8	93.8	544.2	NC	NC	71.3	54.2	47.4	386.8	NC	NC
37	10–Undecenoic acid	112–38–9	NC	S	118.2	67.4	60	600.2	NC	NC	114.6	134	102.3	694.7	NC	NC	96.7	93.8	101.6	582.2	NC	NC
38	N,N–Dimethylbenzylamine	103–83–3	1BC	L	97.4	50	20.6	168	1BC	1BC	98	38.5	19.7	156.2	1BC	1BC	85.9	44.1	14.9	144.9	1BC	1BC
39	Fluoboric acid (hydrogentetrafluoroborate) (48%)	16872–11–0	1BC	L	11.5	4.1	3.5	11.5	1A	1A	18.9	4.1	4.8	18.9	1A	1A	9.6	2.5	2.7	9.6	1A	1A
40	Maleic anhydride	108–31–6	1BC	S	78.8	13	2.7	91.8	1BC	1BC	72.5	10.1	4.3	82.6	1BC	1BC	80.2	6	5.5	86.2	1BC	1BC
41	60/40 octanoic/decanoc acid	68937–75–7	1BC	L	77.4	7.4	2	84.8	1BC	1BC	55	12.4	3.4	67.4	1BC	1BC	103.6	18	7.3	121.6	1BC	1BC
42	55/45 octanoic/decanoc acid	68937–75–7	1BC	L	59.3	18	4.1	77.3	1BC	1BC	68.8	13.6	4.6	82.4	1BC	1BC	103.2	7.6	3	110.8	1BC	1BC

43	65/35 octanoic/decanoic acid	68937–75–7	1BC	L	62.4	8.9	3.4	71.3	1BC	1BC	84.4	7.4	2.7	91.8	1BC	1BC	100.7	8.5	3.5	109.2	1BC	1BC
44	N,N-dimethylisopropylamine	996–35–0	1BC	L	94.3	12.9	8.9	107.2	1BC	1BC	87.7	14.2	8.3	101.9	1BC	1BC	77.6	13.7	9.4	91.3	1BC	1BC
45	Hydrochloric acid (14.4%)	7647–01–0	1BC	L	69.3	5.7	1.9	75	1BC	1BC	63.3	2.2	4.6	65.5	1BC	1BC	65.3	2.5	6.8	67.8	1BC	1BC
46	n-Heptylamine	111–68–2	1BC	L	36.2	50.7	31.9	118.8	1BC	1BC	43.9	25.5	9.7	69.4	1BC	1BC	26.2	22.9	13.3	26.2	1A	1A
47	Octanoic acid (caprylic acid)	124–07–2	1BC	L	16.5	4.1	5.3	16.5	1A	1A	25	6.7	5.3	25	1A	1A	18.2	3.7	3.7	18.2	1A	1A
48	Carvacrol	499–75–2	1BC	L	48.9	23.4	12.1	72.3	1BC	1BC	57.3	26.5	12.5	83.8	1BC	1BC	73.4	31.1	14.7	104.5	1BC	1BC
49	2-tert-Butylphenol	88–18–6	1BC	L	86.3	7.5	12.3	93.8	1BC	1BC	52.9	20.8	9.2	73.7	1BC	1BC	60.7	7.2	9.7	67.9	1BC	1BC
50	Methacrolein	78–85–3	1BC	L	85.4	20.6	0	106	1BC	1BC	122.3	32.6	45.4	154.9	1BC	1BC	92.3	42.9	27	162.2	1BC	1BC
51	Lactic acid	598–82–3	1BC	L	80.2	9.4	6.8	89.6	1BC	1BC	72.3	6.3	4	78.6	1BC	1BC	93.6	8.9	4.2	102.5	1BC	1BC
52	Sodium bisulphate monohydrate	10034–88–5	1BC	S	108	51.5	12.2	171.7	1BC	1BC	102.3	40.9	27.2	170.4	1BC	1BC	88.5	44.3	18.8	151.6	1BC	1BC
53	Glyoxylic acid monohydrate	563–96–2	1BC	S	110.4	22.5	24.6	132.9	1BC	1BC	93.6	40.5	12.3	146.4	1BC	1BC	84.9	27.6	10.8	112.5	1BC	1BC
54	Sodium bisulphate	7681–38–1	1BC	S	100.1	31.7	4	131.8	1BC	1BC	103.7	44.1	10.7	158.5	1BC	1BC	83.4	35.9	14.6	133.9	1BC	1BC
55	Cyclohexylamine	108–91–8	1BC	L	89.8	13.1	8.4	102.9	1BC	1BC	46.8	3.4	18.3	50.2	1BC	1BC	73.9	9.2	10.3	83.1	1BC	1BC
56	2-Methylbutyric acid	600–07–7	1BC	L	80.4	2.2	3.2	82.6	1BC	1BC	35.9	3.9	5.6	39.8	1BC	1A	83	5	3.6	88	1BC	1BC
57	Glycol bromoacetate (85%)	3785–34–0	1BC	L	106.3	33.8	27	140.1	1BC	1BC	70.6	56	30.6	157.2	1BC	1BC	90.1	71.3	16.9	178.3	1BC	1BC
58	3-Methoxypropylamine	5332–73–0	1BC	L	32.8	20.1	12.9	32.8	1A	1A	23.8	15.8	7.9	23.8	1A	1A	38	30	29.8	68	1BC	1BC
59	Allyl bromide	106–95–6	1BC	L	113.9	37.9	8.2	160	1BC	1BC	119.4	17.4	21.9	136.8	1BC	1BC	108.7	21.1	7.7	129.8	1BC	1BC
60	1-(2-Aminoethyl)piperazine	140–31–8	1BC	L	89.5	69.7	26.7	185.9	1BC	1BC	90.9	41.8	5.5	138.2	1BC	1BC	87.2	57.6	27.3	172.1	1BC	1BC
61	Iron(III) chloride	7705–08–0	1BC	S	77.6	–	43.1	–	NC	–	80.4	58.6	50.6	430.8	NC	NC	78	89.6	28.3	195.9	1BC	1BC
62	Phosphoric acid	7664–38–2	1BC	L	65.6	20.6	1.7	86.2	1BC	1BC	113.1	9.8	2.3	122.9	1BC	1BC	67.4	20.8	9	88.2	1BC	1BC
63	Propionic acid	79–09–4	1BC	L	3.2	2.5	3.9	3.2	1A	1A	5.5	11.8	4.3	5.5	1A	1A	5.8	4.3	9.3	5.8	1A	1A
64	Butyric acid	107–92–6	1BC	L	3.8	2.4	6.3	3.8	1A	1A	8	2.8	3.4	8	1A	1A	14.8	4.7	4.6	14.8	1A	1A
65	Boron trifluoride -acetic acid complex	373–61–5	1BC	L	29.1	4	5.9	29.1	1A	1A	71.6	3	2.3	74.6	1BC	1BC	28.9	4	3.1	28.9	1A	1A
66	Ethanolamine	141–43–5	1BC	V	66.2	40.3	20.8	127.3	1BC	1BC	105.7	52.3	20.2	178.2	1BC	1BC	78.7	67.4	10.8	156.9	1BC	1BC
67	Hydrobromic acid (48%)	10035–10–6	1BC	L	15.8	16.4	5.9	15.8	1A	1A	4.1	3.2	2.9	4.1	1A	1A	2.8	4.2	4.6	2.8	1A	1A
68	HCl + sulphuric acid + citric acid (5, 5, 5 wt.%)	–	1BC	L	84.6	1.6	4.1	86.2	1BC	1BC	95.4	19.3	3.3	114.7	1BC	1BC	79.4	32.4	4.5	111.8	1BC	1BC
69	1,2-Diaminopropane	78–90–0	1A	L	37.2	21.2	11.8	58.4	1BC	1BC	33	14.7	8.3	33	1A	1A	32	14.4	13	32	1A	1A
70	Phosphorus tribromide	7789–60–8	1A	L	5.5	2.5	8.1	5.5	1A	1A	8.4	8	1.8	8.4	1A	1A	9.4	15.1	8.6	9.4	1A	1A
71	Boron trifluoride dihydrate	13319–75–0	1A	L	2.4	4.2	2.7	2.4	1A	1A	2.5	2.6	1.9	2.5	1A	1A	4.5	2.9	2.6	4.5	1A	1A

(continued on next page)

Table 9 (*continued*)

72	Acrylic acid	79–10–7	1A	L	1.8	2.7	3.2	1.8	1A	1A	2.4	3.8	3.4	2.4	1A	1A	2.8	2.5	2.9	2.8	1A	1A
73	Formic acid	64–18–6	1A	L	4.3	5.6	9.6	4.3	1A	1A	5.7	4.4	5.8	5.7	1A	1A	7.8	4.8	9.9	7.8	1A	1A
74	Dichloroacetyl chloride	79–36–7	1A	L	5.6	6.3	8.3	5.6	1A	1A	5.8	8.5	10.2	5.8	1A	1A	6.2	10.5	8.1	6.2	1A	1A
75	Silver nitrate	7761–88–8	1A	S	12.1	13.4	14.5	12.1	1A	1A	80.6	4.4	1.2	85	1BC	1BC	66.9	2.5	6.4	69.4	1BC	1BC
76	Phenol	108–95–2	1A	S	29.8	21.8	23.1	29.8	1A	1A	22	18.4	18.5	22	1A	1A	21.4	17.2	17.2	21.4	1A	1A
77	Acetic acid	64–19–7	1A	L	2.4	5.6	3	2.4	1A	1A	4.5	4.7	2.8	4.5	1A	1A	2.9	4.1	2.6	2.9	1A	1A
78	Bromoacetic acid	79–08–3	1A	S	3	2.8	3.5	3	1A	1A	3	2.5	3.7	3	1A	1A	2	2.7	4.3	2	1A	1A
79	N,N–dimethyl–dipropylenetriamine	10563–29–8	1A	L	93.5	55.1	23.3	171.9	1BC	1BC	90.8	45	32	167.8	1BC	1BC	74.4	70.6	28.7	173.7	1BC	1BC
80	Sulphuric acid (98%)	7664–93–9	1A	L	8	13.8	9.7	8	1A	1A	10.5	9.9	10.1	10.5	1A	1A	14.1	13.9	14.4	14.1	1A	1A

Table 10
Contingency table for EpiSkin™ using current PM.

EpiSkin™ using its original PM, 240 predictions (80 chemicals tested 3 times independently)							
In vivo	In vitro predictions						
	1A		1BC		NC		Total
1A	30	83.33%	6	16.67%	0	0.00%	36
1BC	20	21.51%	71	76.34%	2	2.15%	93
NC	0	0.00%	23	20.72%	88	79.28%	111
Total	50		100		90		240
Overall accuracy: 78.75%							

Table 12
Contingency table for SkinEthic™ RHE using current PM.

SkinEthic™ RHE using its original PM, 240 predictions (80 chemicals tested 3 times independently)							
In vivo	In vitro predictions						
	1A		1BC		NC		Total
1A	31	86.11%	5	13.89%	0	0.00%	36
1BC	43	46.24%	43	46.24%	7	7.52%	93
NC	3	2.70%	27	24.32%	81	72.97%	111
Total	77		75		88		240
Overall accuracy: 64.58%							

also shows that a majority of them fulfill this condition. This latter explains why EpiSkin™ current PM presents a high rate (76.34%, see Table 10) of correct predictions for Cat1BC (and low over-prediction rate).

3.3. Results obtained on the basis of PMvar1

PMvar1 has been developed taking into consideration the observations on distribution of cell viability values. Therefore, as we observed that all non-corrosive chemicals are above the cutoffs of v3 = 50% and v60 = 15% it seems logical to keep them under Step 1 of PMvar1 to discriminate corrosive and non-corrosive chemicals. Then under Step 2 the cutoff value of v3 is adjusted to allow the best discrimination possible between Cat1A and Cat1BC. This approach is represented in Figs. 5, 6 and 7 and results are in Tables 14, 15 and 16.

PMvar1 has been tested for EpiDerm™, SkinEthic™ RHE and epiCS®. Prediction rates are expressed over the total number of predictions made. For each of these three methods, the purpose was to improve predictions for Cat1BC, and the overall accuracy, and simultaneously to keep the correct predictions for Cat1A at least at the same level of EpiSkin™.

(1) For EpiDerm™, two cutoff values of x have been tested with PMvar1, x = 20 or 25 (Table 14):

- With x = 20, the correct prediction rate of Cat1BC is 75.27%; the correct prediction rate for Cat1A is 77.78% (below the target of 83.33%) and the overall accuracy is 75.00%.
- With x = 25, the correct prediction rate of Cat1BC is above 70% (70.97%); the correct prediction rate for Cat1A reaches the target value of 83.33% and the overall accuracy is 74.17%.

(2) For SkinEthic™ RHE, the two cutoff values that have been tested with PMvar1 are x = 8 or 18 (Table 15):

- With x = 8, the correct prediction rate of Cat1BC is 68.82%; the correct prediction rate for Cat1A is 77.78% (below the target value of 83.33%) and the overall accuracy is 72.08%.
- With x = 18, the correct prediction rate of Cat1BC is above 61% (61.29%); the correct prediction rate for Cat1A reaches the target value of 83.33%.

(3) For epiCS®, the two cutoff values that have been tested with PMvar1, x = 10 or 15 (Table 16):

- With x = 10, the correct prediction rate of Cat1BC is 65.57%; the correct prediction rate for Cat1A is 79.17% (below the target value of 83.33%) and the overall accuracy is 70.44%.
- With x = 15, the correct prediction rate of Cat1BC is 60.66%; the correct prediction rate of Cat1A is 87.50% thus above the target value of 83.33% and the overall accuracy is 69.81%.

Therefore for EpiDerm™, SkinEthic™ RHE and epiCS®, we were able to find the most appropriated values of x (x = 25; 18 and 15 respectively) in PMvar1 that simultaneously increased the correct prediction rates for Cat1BC and meet the target value of correct prediction rates for Cat1A (see Section 2.2).

3.4. Results obtained on the basis of PMvar2

As for PMvar1, PMvar2 was also used for EpiDerm™, SkinEthic™ RHE and epiCS®, prediction rates are reported as well as the overall accuracy. Figs. 8, 9 and 10 present the distribution of the composite indicator vfin. Tables 17, 18 and 19 present the results for each test method when several cutoff values of

(1) For EpiDerm™, two cutoff values have been tested y = 20 or 25 with z = 115 (Table 17)

- With y = 20, the correct prediction rate for Cat1BC is 75.27%; the correct prediction rate for Cat1A is 77.78% (below the target of 83.33%) and the overall accuracy is 75.83%.
- With y = 25, the correct prediction rate of Cat1BC is slightly above 70%; the correct prediction rate for Cat1A is 83.33% and reaches the target level of EpiSkin™ and the overall accuracy is 75.00%.

(2) For SkinEthic™ RHE, two cutoff values have been tested y = 11 or 18 with z = 130 (Table 18).

- With y = 11, the correct prediction rate of Cat1BC is 65.59%; the correct prediction rate of Cat1A is 80.56% (below the target rate of 83.33%) and the overall accuracy is 71.25%.

Table 11
Contingency table for EpiDerm™ using current PM.

EpiDerm™ using its original PM, 240 predictions (80 chemicals tested 3 times independently)							
In vivo	In vitro predictions						
	1A		1BC		NC		Total
1A	33	91.67%	3	8.33%	0	0.00%	36
1BC	39	41.94%	54	58.06%	0	0.00%	93
NC	3	2.70%	26	23.42%	82	73.87%	111
Total	73		85		82		240
Overall accuracy: 70.41%							

Table 13
Contingency table for epiCS® using current PM.

epiCS® using its original PM, 159 prediction (79 chemicals independently tested twice plus 1 chemical tested once)							
In vivo	In vitro predictions						
	1A		1BC		NC		Total
1A	22	91.67%	2	8.33	0	0.00%	24
1BC	28	45.90	29	47.54%	4	6.56%	61
NC	0	0.00%	21	28.38	53	71.62%	74
Total	50		52	52	57		159
Overall accuracy: 65.41%							

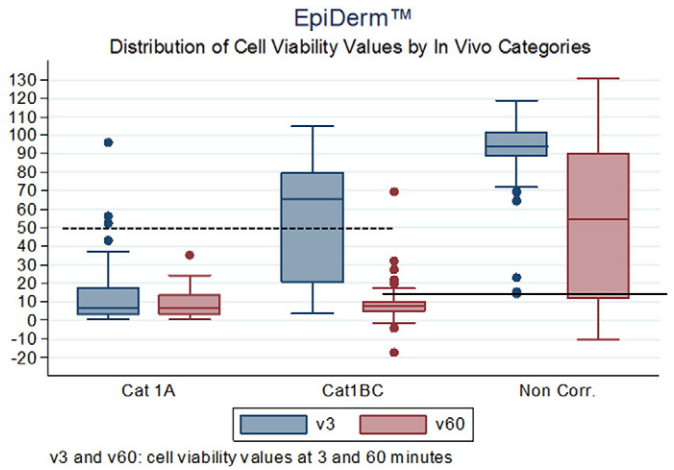

Fig. 1. Distribution of cell viability values in EpiDerm™ (dash line: 50% cutoff for v3; full line: 15% cutoff for v60). Boxplots represent: median (line within box), 25th percentile (lower hinge) and its lower adjacent value (lower adjacent line), 75th percentile (upper hinge) and its upper adjacent value (upper adjacent line), outside values (dots).

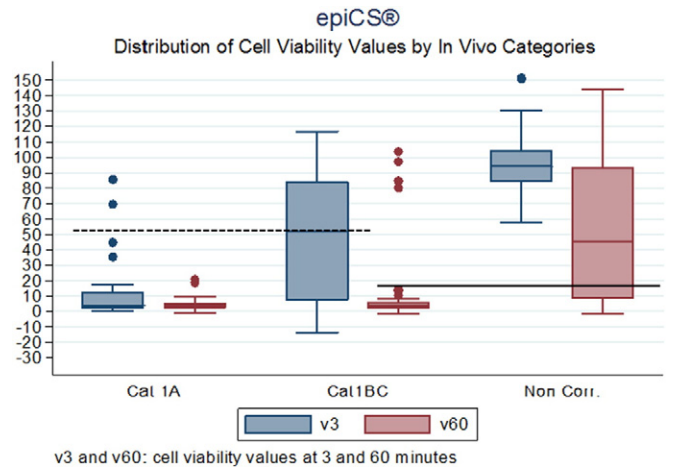

Fig. 3. Distribution of cell viability values in epiCS (dash line: 50% cutoff for v3; full line: 15% cutoff for v60). Boxplots represent: median (line within box), 25th percentile (lower hinge) and its lower adjacent value (lower adjacent line), 75th percentile (upper hinge) and its upper adjacent value (upper adjacent line), outside values (dots).

- With y = 18, the correct prediction rate of Cat1BC is slightly above 61.29%; the correct prediction rate for Cat1A reaches the target level of EpiSkin™ at 83.33% and the overall accuracy is 70.00%.

(3) For epiCS®, two cutoff values have been tested y = 7 or 18 with z = 130 (Table 19)

- With y = 7, the correct prediction rate of Cat1BC is 68.85%; the correct prediction rate of Cat1A is 70.83% (below the target rate of 83.33% from EpiSkin™) and the overall accuracy is 70.44%.
- With y = 18, the correct prediction rate of Cat1BC is slightly below 60%; the correct prediction rate for Cat1A that reaches 91.67% is over the target level of EpiSkin™ and the overall accuracy is 69.81%

When using PMvar2, the most appropriated values of y and z are (x = 25, z = 115) for EpiDerm™ and (x = 18, z = 130) for SkinEthic™ RHE and epiCS®. These values, similarly to what has been done in PMvar1, increased the correct prediction rates for Cat1BC and simultaneously allowed reaching the target value for Cat1A.

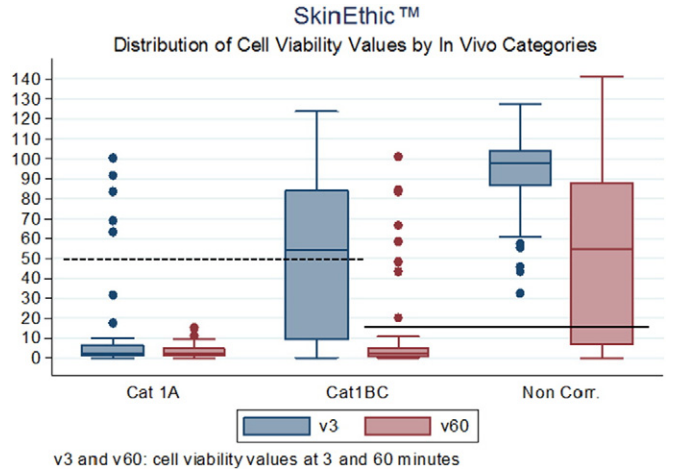

Fig. 2. Distribution of cell viability values in SkinEthic™ RHE (dash line: 50% cutoff for v3; full line: 15% cutoff for v60). Boxplots represent: median (line within box), 25th percentile (lower hinge) and its lower adjacent value (lower adjacent line), 75th percentile (upper hinge) and its upper adjacent value (upper adjacent line), outside values (dots).

4. Discussion

4.1. Possible limitations

Results for EpiDerm™ and SkinEthic™ RHE are derived from 3 runs while those for epiCS® are derived from 2 runs. Therefore there might be more uncertainty for results derived from epiCS®. However since results derived from epiCS® are consistent with those from the two other methods and fall in the same range of values, they still could be considered as representative of the performance & changes observed regarding this method.

Additionally, two other factors might influence the final outcomes: coloring and/or MTT-reducing chemicals, as they intrinsically have an impact on OD. In 2012 and 2013 the OECD experts considered that these factors did not influence the final outcomes. Our investigation is focused on prediction models only, and does not assess how predictions could be influenced by physical–chemical properties e.g., coloring chemicals and/or MTT-reducing chemicals, physical state, chemical category. This can be performed using specific statistical modeling and may be the subject of another paper. However it is noteworthy that, as the set of chemicals used by all the RhE methods of this study is the same, comparison can be done between methods when their PMs are

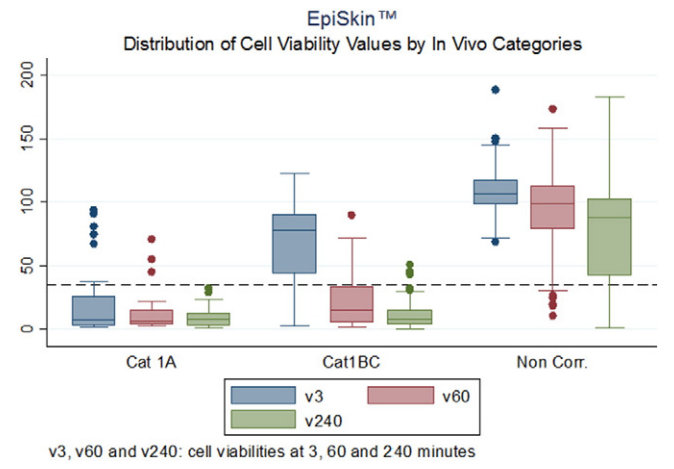

Fig. 4. Distribution of cell viability values in SkinEthic™ (dash line: 35% cutoff). Boxplots represent: median (line within box), 25th percentile (lower hinge) and its lower adjacent value (lower adjacent line), 75th percentile (upper hinge) and its upper adjacent value (upper adjacent line), outside values (dots).

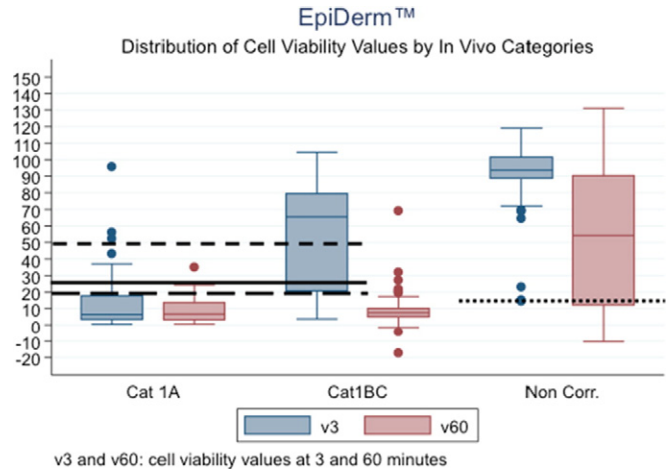

Fig. 5. Distribution of cell viabilities at 3 and 60 min for EpiDerm™. Dash line: cut off of 50% for v3 in original PM, dot line: cutoff of 15% for v60 in original PM and PMvar1, continuous line: 25% cutoff in PMvar1 (x = 25); long-dash line 20% cutoff in PMvar (x = 20). Boxplots represent: median (line within box), 25th percentile (lower hinge) and its lower adjacent value (lower adjacent line), 75th percentile (upper hinge) and its upper adjacent value (upper adjacent line), outside values (dots).

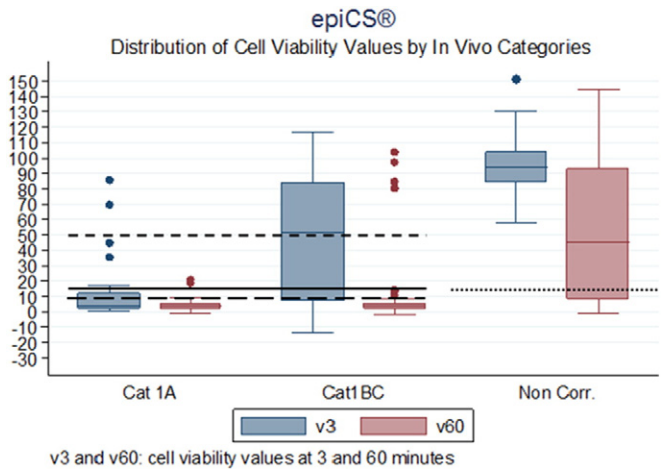

Fig. 7. Distribution of cell viabilities at 3 and 60 min for epiCS®. Dash line: cut off of 50% for v3 in original PM, dot line: cutoff of 15% for v60 in original PM and PMvar1, continuous line: 15% cutoff in PMvar1 (x = 15); long-dash line 10% cutoff in PMvar (x = 10). Boxplots represent: median (line within box), 25th percentile (lower hinge) and its lower adjacent value (lower adjacent line), 75th percentile (upper hinge) and its upper adjacent value (upper adjacent line), outside values (dots).

changed, and therefore be able to assess the impact of these changes both within a given method and between methods.

4.2. PMvar1 and PMvar2 both improved the predictions for Cat1BC and the overall accuracy in comparison to the original PM

For Epiderm™, SkinEthic™ RHE and epiCS® both PMvar1 and PMvar2 improved the predictions for Cat1BC and the overall accuracy in comparison to the original prediction model. Simultaneously, the target value of correct prediction rates for Cat1A (i.e., 83.33%) was maintained in EpiDerm™ and SkinEthic™ RHE and surpassed in epiCS®. This was obtained by selecting the most appropriated cutoff values of x in PMvar1 and y & z in PMvar2. These results are summarized in Table 20.

These results show that the extent of changes in predictions made by PMvar1 and PMvar2 are comparable for the three tissue models. We can confirm this by using the kappa coefficient when the most appropriated values of x, y and z, for each test method (Sections 3.3 and 3.4), are used.

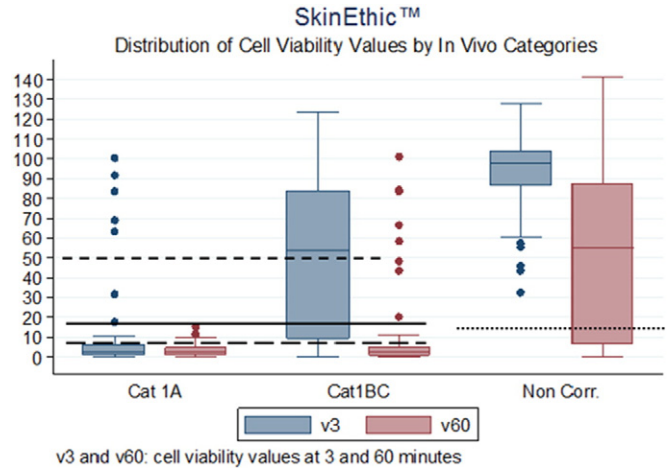

Fig. 6. Distribution of cell viabilities at 3 and 60 min for SkinEthic™ RHE. Dash line: cut off of 50% for v3 in original PM, dot line: cutoff of 15% for v60 in original PM and PMvar1, continuous line: 18% cutoff in PMvar1 (x = 18); long-dash line 8% cutoff in PMvar (x = 8). Boxplots represent: median (line within box), 25th percentile (lower hinge) and its lower adjacent value (lower adjacent line), 75th percentile (upper hinge) and its upper adjacent value (upper adjacent line), outside values (dots).

This is presented in Table 21. It allows one to perform a quantitative evaluation of the agreement between PMvar1 and PMvar2.

For Epiderm™, SkinEthic™ RHE and epiCS® the respective values of agreement/kappa coefficient are 99.17%/0.987, 100%/1.000 and 99.37%/0.990 (Table 21). As all those values are close to 100% for the agreement and 1.000 for the Kappa coefficient, they support the fact that PMvar1 and PMvar2 provide comparable results as regards these 3 methods.

4.3. Specific advantages of PMvar2

4.3.1. PMvar2 additionally helps to perform ROC analysis

Briefly, a ROC curve is a plotting graph where x-axis and y-axis are respectively represented by (1-Specificity) and Sensitivity when monotonic variation of the cutoff value is applied for yes/no predictions (Fawcett, 2006). The theoretical best performance is obtained when (1-Specificity) value is 0 and simultaneously Sensitivity value is 1 and therefore the area under ROC curve (AUROC) should be as close as possible to the value of 1 (Fawcett, 2006).

ROC curves are mostly obtained on the basis of a single variable (however logistic regression modeling allow performing ROC analysis on the basis of several dependent variables, see Section 2.2, sub-section "Obtaining of ROC curves when PMvar2 is applied"), and thus prediction models using different classification variables—i.e., different cell viabilities—cannot easily lead to obtaining ROC curves. The obtaining of

Table 14
Contingency table for EpiDerm™ using PMvar1.

EpiDerm™, PMvar1 x = 20 (not in italics) or 25 (*in italics*) 240 predictions (80 chemicals tested 3 times independently)							
In vivo	In vitro predictions						
	1A		1BC		NC		Total
1A	28	77.78%	8	22.22%	0	0.00%	36
	30	*83.33%*	*6*	*16.67%*	*0*	*0.00%*	36
1BC	23	24.73%	70	75.27%	0	0.00%	93
	27	*29.03%*	*66*	*70.97%*	*0*	*0.00%*	93
NC	2	1.80%	27	24.32%	82	73.87%	111
	3	*2.70%*	*26*	*23.42%*	*82*	*73.87%*	111
Total	53		105		82		240
	60		*97*		*82*		240
Overall accuracy: 75.00% *74.17%*							

Table 15
Contingency table for SkinEthic™ RHE using PMvar1.

SkinEthic™ RHE, PMvar1 x = 8 (not in italics) or 18 (*in italics*) 240 predictions (80 chemicals tested 3 times independently)						
	In vitro predictions					
	1A		1BC		NC	Total
28	77.78%	8	22.22%	0	0.00%	36
30	*83.33%*	*6*	*16.67%*	*0*	*0.00%*	36
22	23.66%	64	68.82%	7	7.53%	93
29	*31.18%*	*57*	*61.29%*	*7*	*7.53%*	93
0	0.00%	30	27.03%	81	72.97%	111
0	*0.00%*	*30*	*27.03%*	*81*	*72.97%*	111
50		102		88		240
59		*93*		*88*		240
Overall accuracy 72.08% *70.00%*						

the ROC curves, regarding the different types of predictions, has been already explained in Section 2.2 on data analysis. PMvar2 renders this ROC analysis possible, which represents a significant advantage in comparison to, for instance, the current PM.

Figs. 11, 12 and 13 display the ROC curves and areas under ROC curves for the four test methods with regard to predictions: Cat1A versus (Cat1BC and Non-Corr.); Cat1BC versus (Cat1A and Non-Corr.) and Non-Corr. versus (Cat1A and Cat1BC). The results of the ROC analysis are the following:

(1) For predictions of Cat1A versus other categories (Fig. 11) the ROC curves and the corresponding AUROCs are similar. The same conclusion is drawn for predictions Non-Corr. versus other categories (Fig. 13). For prediction type 'Cat1A versus other categories', the AUROCs are all close or above 0.90 i.e., respectively for EpiSkin™, EpiDerm™, SkinEthic™ RHE, and epiCS®: 0.9277; 0.9326; 0.9130 and 0.8934.

- For predictions 'Non-Corr. versus other categories', the AUROCs are all above 0.93 i.e., respectively for EpiSkin™, EpiDerm™, SkinEthic™ RHE, and epiCS®: 0.9711; 0.9723; 0.9309 and 0.9434.
- Moreover a Chi-square test comparing the AUROCs presents a p-value of 0.8785 for predictions 'Cat1A versus other categories' and a p-value of 0.4552 for 'Non-Corr. versus other categories'. Therefore for these two types of predictions, the null hypothesis of similar performance of the four methods cannot be excluded i.e., performances are not significantly different.

(2) For prediction 'Cat1BC versus other categories' (Fig. 12), the AUROCs present contrasted values i.e., respectively for EpiSkin™, EpiDerm™, SkinEthic™ RHE, and epiCS®: 0.9177; 0.8035; 0.7044 and 0.6662. The Chi-square test comparing the AUROCs presents a p-value < 0.0001, the null hypothesis of equality of the four areas is thus excluded. These areas are thus significantly different. This global result can be assessed further by pairwise comparison of the areas with Bonferroni correction. For all possible pairs of areas, significant differences are observed between EpiSkin™ and SkinEthic™ RHE (p-value of 0.0013); EpiSkin™ and epiCS® (p-value of 0.0003). The p-value for comparison of EpiSkin™ and EpiDerm™ is as the limit of the significance, just above the p-value of 0.05 for which the null hypothesis of identical AUROCS cannot be reject (p-value of 0.0565).

Table 16
Contingency table for epiCS® using PMvar1.

epiCS®, PMvar1 x = 10 (not in italics) or 15 (*in italics*) 159 predictions (79 chemicals independently tested twice plus 1 chemical tested once)						
	In vitro predictions					
	1A		1BC		NC	Total
19	79.17%	5	20.83%	0	0.00%	24
21	*87.50%*	*3*	*12.50%*	*0*	*0.00%*	24
17	27.87%	40	65.57%	4	6.56%	61
20	*32.79%*	*37*	*60.66%*	*4*	*6.56%*	61
0	0.00%	21	28.38%	53	71.62%	74
0	*0.00%*	*21*	*28.38%*	*53*	*71.62%*	74
36		66		57		159
41		*61*		*57*		159
Overall accuracy: 70.44% *69.81%*						

4.3.2. PMvar2 remains easily interpretable in the regulatory context

In both chemical and test method regulations, regulators focus on the possible best regulatory use of the test methods according to their performance and results that they provide, especially with regard to human health protection. They have to address prediction models, and those should therefore also be understandable and clear.

There are other possibilities to make predictions using simultaneously different values of cell viabilities (e.g., v3 and v60). One of them is the logistic regression and that also allows ROC analysis and thus finding the best possible cutoff. However, this type of analysis might not be working for all sub-categories. For example in the case of EpiSkin™, when performing a logistic modeling (not shown) for prediction of the sub-category Cat1BC, all dependent variables v3, v60 and v240 return significant coefficients in the model. Therefore it is not easy to determine which cell viability to consider for changing the cutoff.

Furthermore, the interpretation of such logistic modeling may remain uneasy for regulators. They may face the challenge of developing or amending regulation relying on mathematical models rather than directly on threshold of cell viabilities. In contrast the use of the composite cell indicator value used in PMvar2 may help getting round these difficulties.

4.4. Revising TG 431 by inclusion of PMvar1 and/or 2: choosing the best possible one

In Section 4.2 we showed that PMvar1 and PMvar2 performed similarly. Therefore, the choice between these two PMs cannot be based on their performance but rather on their specific advantages.

Usually the predictive performance of a test method is assessed by examining contingency tables and predictions, but it is noteworthy that those are obtained *only for fixed values of the cutoff chosen*. In contrast, the ROC analysis, which is enabled by PMvar2, has the unique advantage to help understanding the predictive performance for *all possible cutoffs*. PMvar2 may therefore represent a potential additional tool offered to regulators, and although apparently more complex it remains interpretable in the regulatory context (Section 4.3.2). Nonetheless, PMvar2 is more likely to be used for academic purposes or for advanced evaluation of the method (e.g., during a peer review process of the test method). For instance, when considering one tissue model, it is possible to compare the areas under ROC curves between runs. In this comparison exercise, the absence of statistically significant difference would not provide additional information (the null hypothesis of equal areas under ROC cannot be rejected); while finding a significant difference between runs would mean that, at least, one of them performs differently from the two others and should trigger more investigation (e.g., whether compliance with the protocol has been respected in all runs). PMvar2 allows performing a more sophisticated statistical analysis, which is a marked advantage.

PMvar1, in contrast to PMvar2, does not allow such advanced analysis but may be easily understood by the regulators. Although the two-step approach of PMvar1 might seem to be a complication, it might

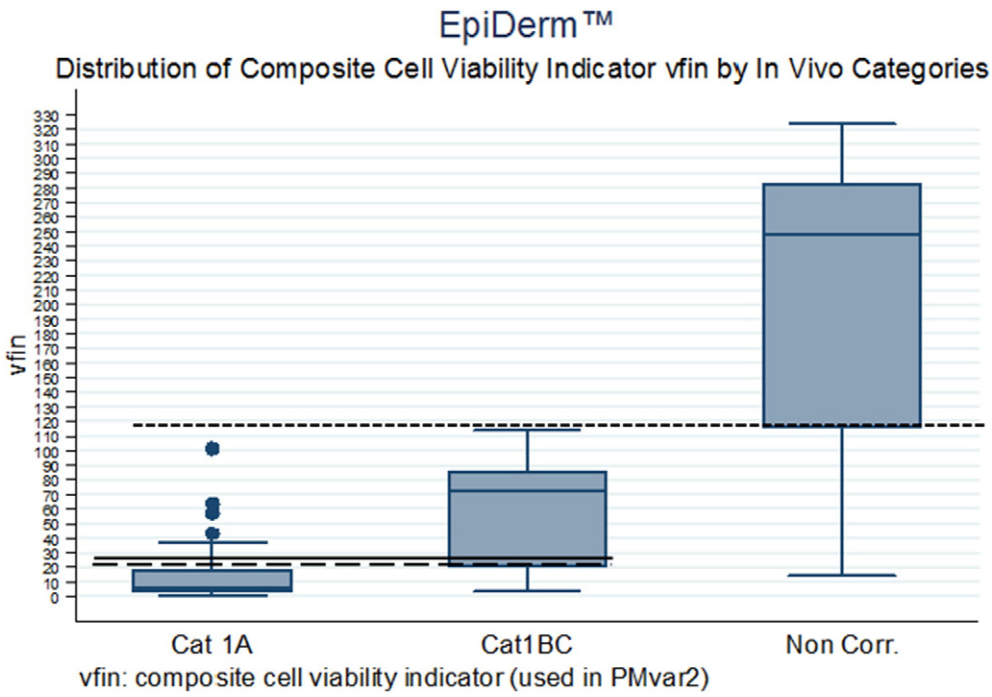

Fig. 8. Distribution of composite cell viability indicator vfin for EpiDerm™ for PMvar2. Dot line: cutoff of vfin = 115 for vfin, continuous line: cutoff vfin = 25; long-dash line of vfin = 20. Boxplots represent: median (line within box), 25th percentile (lower hinge) and its lower adjacent value (lower adjacent line), 75th percentile (upper hinge) and its upper adjacent value (upper adjacent line), outside values (dots).

also remain familiar. Therefore, from a practical point of view, it is likely that the inclusion of PMvar1 in TG 431 would require a less quantity of work.

For regulatory purposes, we recommend that PMvar1 is used for practical reasons. For academic or more advanced evaluation of the test methods, we rather recommend that PMvar2 as it allows the types of analysis presented above.

5. Conclusion

Reconstructed human Epidermis (RhE) test methods are playing a prominent role in topical toxicology and for risk assessment purposes, especially with regard to the development of Integrated Approaches on Testing and Assessment (OECD, 2014a) and the ban of animal use in cosmetic area in Europe since March 2013.

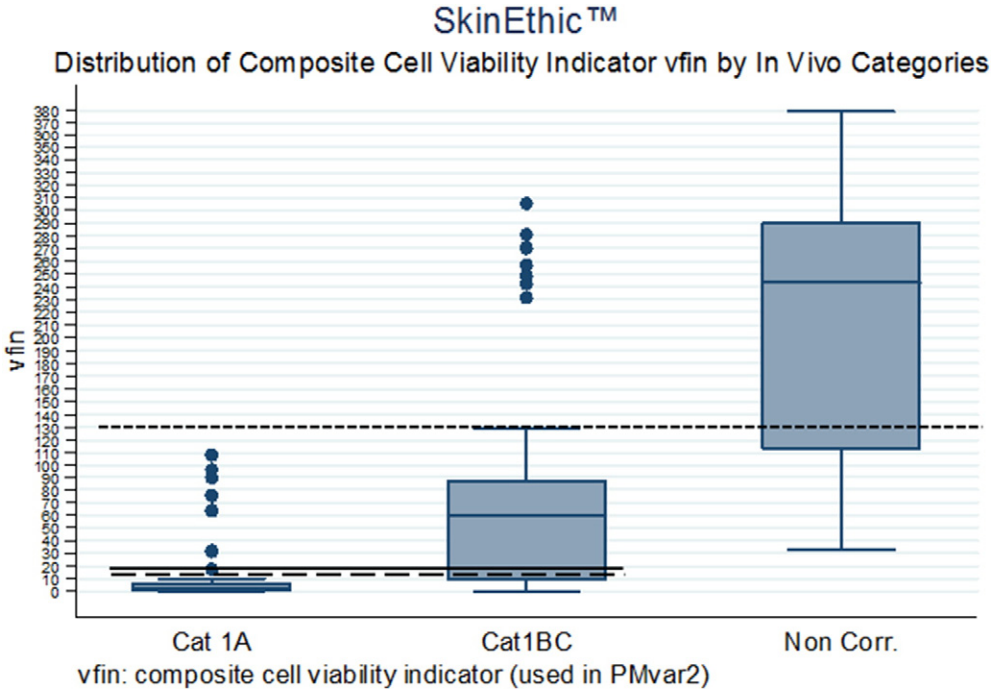

Fig. 9. Distribution of composite cell viability indicator vfin for SkinEthic™ RHE for PMvar2. Dot line: cutoff of vfin = 130 for vfin, continuous line: cutoff vfin = 18; long-dash line of vfin = 11. Boxplots represent: median (line within box), 25th percentile (lower hinge) and its lower adjacent value (lower adjacent line), 75th percentile (upper hinge) and its upper adjacent value (upper adjacent line), outside values (dots).

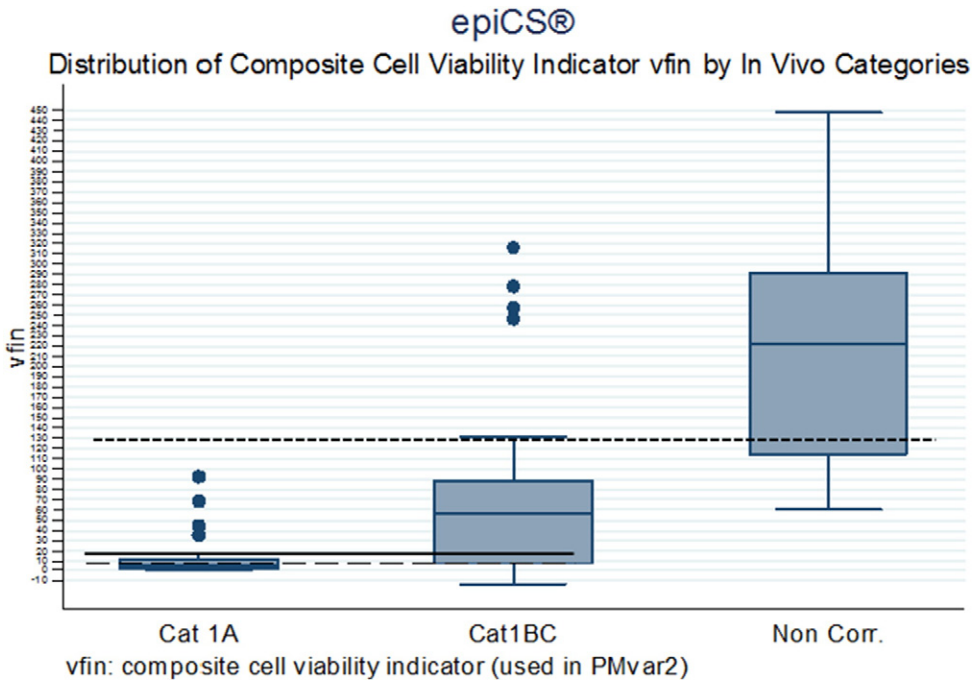

Fig. 10. Distribution of composite cell viability indicator vfin for ePiCS® for PMvar2. Dot line: cutoff of vfin = 130 for vfin, continuous line: cutoff vfin = 18; long-dash line of vfin = 7. Boxplots represent: median (line within box), 25th percentile (lower hinge) and its lower adjacent value (lower adjacent line), 75th percentile (upper hinge) and its upper adjacent value (upper adjacent line), outside values (dots).

For the skin corrosion endpoint, predictions derived from the use of these methods shall be sufficiently protective in terms of human health, i.e., shall at least be able to discriminate corrosive chemicals from non-corrosive ones. Additionally, for industrial purposes and cost considerations, especially with regard to the transport of chemicals, discrimination within corrosive chemicals into sub-categories required in regulation is of importance as well, providing prediction types Category 1A versus Category 1BC versus non-corrosive, on the basis of UN GHS categories.

The last version of OECD Test Guideline No. 431 allows these types of sub-categorization for four methods, EpiDerm™; EpiSkin™ and SkinEthic™ RHE, and epiCS®. However the prediction models included in this TG for EpiDerm™, SkinEthic™ RHE and epiCS® result in quite high over-prediction rates of Cat1BC chemicals that are over-predicted as Category 1A, while they present high correct predictions rates of Category 1A and non-corrosive chemicals. For EpiSkin™, predictions provided can be used straightaway, in contrast to the three other methods.

Our analysis shows that the cutoff value of 50% cell viability at 3 min used in EpiDerm™, SkinEthic™ RHE and epiCS® to discriminate between Cat1A and Cat1BC is the main factor in causing this high over-prediction rate. Switching from the original PM to the novel PMs (PMvar1 or PMvar2) allow to obtain higher correct predictions for Cat1BC in a range of 60–70%. Correct predictions for 1A are slightly decreased, in parallel, but remain at least at the level of the EpiSkin™ SCT. This slight decrease occurs in a much lower extent than the increase of correct predictions for 1BC. Additionally, the overall accuracy is also increased, for EpiDerm™ from around 70% initially to around 75%, for SkinEthic™ RHE and epiCS® from around 65% initially to around 70%.

The composite indicator, vfin, used in PMvar2 presents the advantages (i) to be easier to use, (ii) allow ROC analysis, since this analysis was not straightaway forward with the original PM or PMvar1 and (iii) remains accessible to regulators compared to the logistic modeling which is a complex approach.

Table 17
Contingency table for EpiDerm™ using PMvar2.

EpiDerm™, PMvar2 y = 20 (not in italics) or *25* (*in italics*) and z = 115 240 predictions (80 chemicals tested 3 times independently)							
In vivo	In vitro predictions						
	1A		1BC		NC		Total
1A	28	77.78%	8	22.22%	0	0.00%	36
	30	*83.33%*	*6*	*16.67%*	*0*	*0.00%*	36
1BC	23	24.73%	70	75.27%	0	0.00%	93
	27	*29.03%*	*66*	*70.97%*	*0*	*0.00%*	93
NC	2	1.80%	25	22.52%	84	75.66%	111
	3	*2.70%*	*24*	*21.62%*	*84*	*75.66%*	111
Total	53		103		84		240
	60		*96*		*84*		240
Overall accuracy: 75.83% *75.00%*							

Table 18
Contingency table for SkinEthic™ RHE using PMvar2.

SkinEthic™ RHE, PMvar2 y = 11 (not in italics) or *18* (*in italics*) and z = 130 240 predictions (80 chemicals tested 3 times independently)						
	In vitro predictions					
	1A		1BC		NC	Total
29	80.56%	7	19.44%	0	0.00%	36
30	*83.33%*	*6*	*16.67%*	*0*	*0.00%*	36
25	26.88%	61	65.59%	7	7.53%	93
29	*31.18%*	*57*	*61.29%*	*7*	*7.53%*	93
0	0.00%	30	27.03%	81	72.97%	111
0	*0.00%*	*30*	*27.03%*	*81*	*72.97%*	111
54		98		88		240
59		*93*		*88*		240
Overall accuracy 71.25% *70.00%*						

Table 19
Contingency table for epiCS® using PMvar2.

epiCS®, PMvar2 y = 7 (not in italics) or *18* (*in italics*) and z = 130 159 predictions (79 chemicals independently tested twice plus 1 chemical tested once)						
	In vitro predictions					Total
	1A		1BC		NC	
17	79.17%	7	29.17%	0	0.00%	24
22	*91.67%*	*2*	*8.33%*	*0*	*0.00%*	24
14	27.87%	42	68.85%	5	8.20%	61
20	*32.79%*	*36*	*59.02%*	*5*	*8.20%*	61
0	0.00%	21	28.38%	53	71.62%	74
0	*0.00%*	*21*	*28.38%*	*53*	*71.62%*	74
31		70		58		159
42		*59*		*58*		159
Overall accuracy: 70.44% *69.81%*						

Table 20
Variations of correct predictions observed in comparison to the original prediction model. The numbers represents the difference of correct prediction rates, for each sub-category, between PMvar1 or PMvar2 and the original prediction model.

Tissue model	Sub-category considered	PMvar1 with cutoff value of	PMvar2 with cutoff value of
EpiDerm™		*x = 25*	*y = 25, z = 115*
	Cat1BC	+12.91%	+12.91%
	Cat1A	−8.34%	−8.34%
	Overall accuracy	+4.59%	+4.59%
SkinEthic™ RHE		*x = 18*	*y = 18, z = 130*
	Cat1BC	+15.05%	+12.78%
	Cat1A	−2.78%	−2.78%
	Overall accuracy	+5.42%	+5.42%
epiCS®		*x = 15*	*y = 18, z = 130*
	Cat1BC	+13.12%	+11.48%
	Cat1A	−4.17%	0.00%
	Overall accuracy	+4.40%	+4.40%

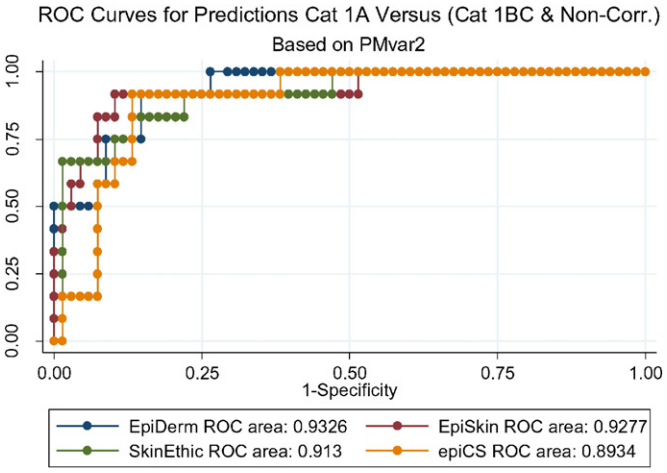

Fig. 11. ROC curves and areas under ROC for prediction Cat1A versus (combined) other categories.

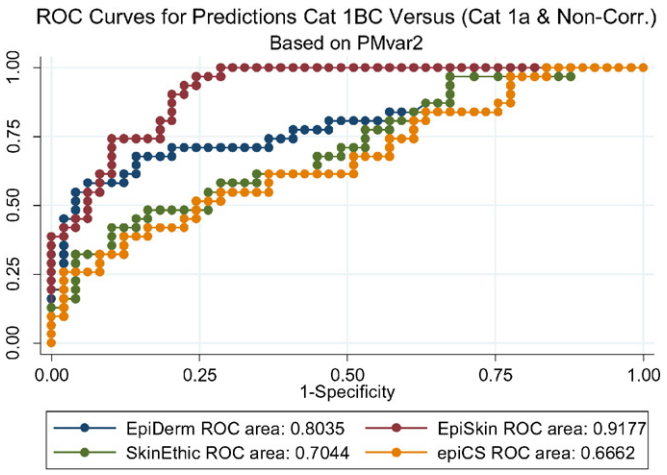

Fig. 12. ROC curves and areas under ROC for prediction Cat1BC versus (combined) other categories.

For all the four methods considered, including EpiSkin™, ROC analysis performed was consistent with their abilities to sub-categorize chemicals as they appear in their respective 3 × 3 contingency tables (when using PMvar2). EpiDerm™, SkinEthic™ RHE and epiCS® ROC curves are consistent with their good abilities for identifying Cat1A and non-corrosive chemicals and increased ability to identify Cat1BC; while EpiSkin™ ROC curves are consistent with its good abilities for identifying Cat1A, and non-corrosive chemicals, as well as greater abilities to identify Cat1BC chemicals.

Overall, for EpiDerm™, SkinEthic™ RHE and epiCS® prediction models regarding sub-categorization of corrosive can be significantly improved by changing of PM. These results show the key role played by PMs of RhE methods for skin corrosion endpoint, and support the fact that they should be carefully adapted for the intended regulatory use. It ultimately supports the need for a possible revision of OECD TG 431 with regard to sub-categorization of chemicals performed by these three methods. PMvar2 has the unique advantage to enable ROC analysis and to permit advanced statistics and easier comparison of the predictive capacities of the methods. Although we showed that PMvar1 and PMvar2 have similar performances and provide comparable predictions, in the regulatory context we think that PMvar1 is easier to use and be understood. Therefore it is our opinion that PMvar1 should be implemented in TG 431.

Conflict of interest statement

The authors declare no conflicts of interest.

Table 21
Agreement and Kappa coefficient between PMvar1 and PMvar2 for each test method.

EpiDerm™						SkinEthic™ RHE						epiCS®					
		PMvar2						PMvar2						PMvar2			
		Cat1A	Cat1BC	NC	Total			Cat1A	Cat1BC	NC	Total			Cat1A	Cat1BC	NC	Total
PMvar1	Cat1A	60	0	0	56	PMvar1	Cat1A	59	0	0	59	PMvar1	Cat1A	41	0	0	41
	Cat1BC	0	96	2	102		Cat1BC	0	93	0	93		Cat1BC	0	60	0	60
	NC	0	0	82	82		NC	0	0	88	88		NC	0	1	57	58
	Total	60	96	84	240		Total	59	93	88	240		Total	41	61	57	159
Agreement: 99.17% Kappa: 0.987						Agreement 100.00% Kappa: 1.00						Agreement: 99.37% Kappa: 0.990					

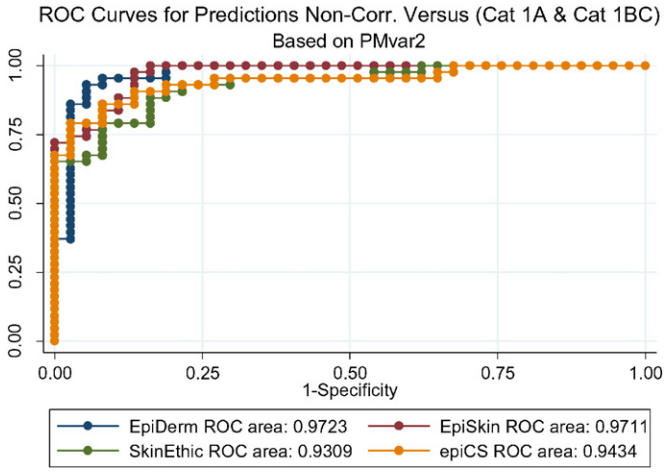

Fig. 13. ROC curves and areas under ROC for prediction Non-Corr. versus (combined) other categories.

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