An Evaluation of the Reconstructed Human Epidermis (RhE) Method for Predicting Skin Corrosivity of Chemical Products with Extreme Acid pH

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ABSTRACT

The purpose of this analysis was to evaluate the Reconstructed Human Epidermis (RhE) model as an *in vitro* method to predict skin corrosivity (OECD 431) for acid products with extreme pH (≤2) when compared with *in vivo* data and the AISE Method (The Worst Case Table) of classification. Extreme pH can be a useful predictor of irritation but may lead to over classification in weakly buffered systems. Our objective was to determine whether the RhE method could accurately identify corrosive and non-corrosive acid products. When compared with the *in vivo* data, 4/7 products tested using the RhE method predicted the same skin classification. The skin classification of the remaining three formulas was over-predicted when compared with the *in vivo* data. There were no products for which the RhE under-predicted the skin classification when compared to the *in vivo* results. When compared with The AISE Method (which considers the results of the EU conventional method calculation and pH/acid reserve), 8/23 products tested using a RhE method predicted the same skin classification. The skin classification of the remaining fifteen formulas was over-predicted when compared with the AISE Method. There were no products in which the RhE under-predicted the skin classification when compared to the AISE method. Overall, the RhE did not reliably identify non-corrosive formulations when compared to either the *in vivo* data or the AISE Method. This presents significant challenges under hazard classification guidelines such as the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), which requires testing with a validated *in vitro* method to confirm a non-corrosive classification for an extreme pH product.

Introduction

Extreme pH is often used as a predictor of irritation but may lead to over classification in weakly buffered systems. For example, 0.04% NaOH has a pH of 12 and 0.05% H_2SO_4 has a pH of 2, neither of which is corrosive. Consideration of acid/alkaline reserve in addition to pH using the Young Method (1) has been a common industry practice in Europe for decades, where it is recognized in relevant classification and labeling regulations. The EU Dangerous Substances Directive (2) indicates that "a substance or a preparation should also be considered corrosive if the result can be predicted, for example from strongly acid or alkaline reactions indicated by pH<2 or >11.5. However, where extreme pH is the basis for classification, acid/alkali reserve may also be taken into consideration."

Following the introduction of the Young method (1), Diversey, Inc. (DI) and other cleaning product manufacturers in Europe classified their extreme pH products for dermal irritation using the more conservative result of the following classification methods:

- The Conventional Calculation Method (CCM) in accordance with the Dangerous Preparations Directive (1999/45/EC) (3)
- The pH/Alkali/Acid Reserve method (1)

This process has provided conservative classifications with a successful market history of protecting consumers and employees in the workplace without the use of animals. The approach was formally adopted for use by the member companies of the European Soap and Detergent Trade Association, Association Internationale de la Savonnerie, de la Détergence et des Produits d'Entretien (AISE) in 2004. This method of classification is referred to as the AISE Method (The Worst Case Table) (4).

The 28th ATP of the Dangerous Substances Directive (2001/59/EC) indicated that a valid *in vitro* test should be used to confirm non-corrosive classifications when using the pH and acid/alkaline reserve methods. This has been incorporated into the GHS Guidelines (5). This latter addition was also introduced for preparations via an ATP to the Dangerous Preparations Directive (1st ATP of the DPD, 2001/60/EC).

In response, DI began a project to evaluate results of the RhE method vs. the results of historical *in vivo* testing and the AISE Method of classification for cleaning product mixtures containing acids and other materials.

Methods & Materials

- Test materials: acids and other chemicals formulated as cleaning products (bath & bowl cleaners, descalers, and machine ware wash detergents), requiring dilution prior to use
- 7 highly acid formulas (pH range from 0.81 to 2.25) with *in vivo* data for classification
- 23 highly acid formulas (pH range from 0.5 to 2.25) were tested in either the EpiDerm™ or EpiSkin™ *in vitro* dermal corrosivity assays (OECD431)
- The *in vivo* studies followed protocols established by either FHSA (24 hour occluded patch) or OECD 404 (4 hour semi-occluded patch)
- All *in vivo* testing was historical; no new *in vivo* studies were conducted
- pH and acid reserve were measured (acid reserve: %NaOH / 100 mL to pH=4) (6)
- All products were classified for dermal irritation/corrosivity using the EC conventional method calculation (3) and the Young Method (5)
- 15 mixtures were tested in the EpiDerm™ corrosivity assay (OECD 431) for 3 and 60 minutes. Relative viabilities of <50% after 3 minutes and/or <15% after 60 minutes were predicted corrosive.
- 8 formulas were tested in the EpiSkin™ corrosivity assay (OECD 431) for 3 minutes, and 1 and 4 hours. Relative viabilities of <35% after 3 minutes, 1 or 4 hours exposure were predicted corrosive.

RESULTS

EpiDerm™ and EpiSkin™ assay results from 2003 to 2010

Mixture ID	pH	EpiDerm™ Results[1] % Viability 3 min	60 min	EpiDerm™ results / Classification
Acid 1	1.4	83.6	8	Corrosive
		94.6	89.8	Non-Corrosive
Acid 2	1.6	90	52.4	Non-Corrosive
Acid 3	2.1	97.5	103.9	Non-Corrosive
Acid 4	1.3	91.5	9.6	Corrosive
Acid 7	0.9	70.8	4.4	Corrosive
Acid 8	0.7	39.8	2.1	Corrosive
Acid 9	0.9	71.1	3.6	Corrosive
Acid 10	2.3	93.4	85.1	Non-Corrosive
Acid 29	0.9	79.9	2.4	Corrosive
Acid 30	1.1	69.4	2.1	Corrosive
Acid 31	0.9	89.2	6	Corrosive
Acid 32	2	102.6	37	Non-Corrosive
Acid 34	1.7	95.9	66	Non-Corrosive
Acid 35	2	58.3	3.6	Corrosive
Acid 36	1.7	103.1	7.6	Corrosive

[1]Relative viability of <50% after the 3-minute exposure, or ≤15% after the 60-minute exposure is predicted to be corrosive

Mixture ID	pH	EpiSkin™ Results[1] % Cell Viability 3 min	1 hr	4 hr	Conclusion
Acid 37	1.6	97	99	60	Non-Corrosive
Acid 38	1.5	98.7	87	74.7	Non-Corrosive
Acid 39	0.5	96.7	0.3	2	Corrosive
Acid 40	1.7	95.7	91	< 1.5	Corrosive
Acid 41	0.8	95	2	1.67	Corrosive
Acid 42	0.5	96.3	14.3	1.3	Corrosive
Acid 43	< 1	97.3	49	1.3	Corrosive
Acid 44	1.9	98.3	98.3	97.3	Non-Corrosive

[1]Relative viability of <35% after the 3 minute, 1 hour or 4 hour exposure is predicted to be corrosive. A chemical is not predicted to be corrosive if the cell viability is > 35% after the 4 hour exposure.

In Vivo Results

- 6 of 7 formulas tested *in vivo* were found to be non-corrosive and were no more than mildly irritating to skin (not classified according to GHS).
- 1 of 7 formulas was found to be corrosive *in vivo*
- The acid reserve for the 6 non-corrosives ranged from 0.025 to 4.34% NaOH/100 mL to pH=4
- The acid reserve for the corrosive formula was 3.59% NaOH/100 mL to pH=4

AISE Prediction versus *In Vivo*

- 7 of 7 formulas were predicted to be non-corrosive using the AISE method.
- 6 of 7 formulas were predicted the same in both the AISE and *in vivo* methods
- 1 of 7 formulas was "under predicted" compared to *in vivo* testing.
- The acid reserve for the 7 products predicted to be non-corrosive in the AISE Method ranged from 0.025 to 4.34% NaOH/100 mL to pH=4

RhE versus *In Vivo*

- 4 of 7 formulas showed concordant predictions between the RhE and *in vivo* methods (1 was corrosive and 3 were non-corrosive)
- The acid reserve for the 3 formulas predicted to be non-corrosive in the RhE method ranged from 0.025 to 1.78% NaOH/100 mL to pH=4
- The acid reserve for the 4 products predicted to be corrosive in the RhE method ranged from 2.65 to 4.34% NaOH/100 mL to pH=4

Mixture ID	Acid Type	Acid (% Active)	Surfactant (% Active)	Physical parameters: pH	Acid Reserve	Classification for Skin: EC CCM	Reserve	AISE	*RhE*	*In Vivo*
Acid 1	Oxalic Acid Dihydrate	30.5	0	1.35	3.94	Not Classified	Irritant	Non Corrosive	Corrosive	Non Corrosive
Acid 2	Phosphoric	5.1	2.17	1.6	1.78	Not Classified	Not classified	Non Corrosive	Non-corrosive	Non Corrosive
Acid 3	Acrylic	4.4	3.6	2.05	0.025	Not classified	Not classified	Non Corrosive	Non-corrosive	Non Corrosive
Acid 4	Oxalic Acid Dihydrate Phosphoric	3 8.5	6	1.3	3.59	Irritant	Irritant	Non Corrosive	Corrosive	Corrosive
Acid 7	Citric	20	12	0.89	5.15	Irritant	Irritant	Non Corrosive	Corrosive	Not tested
Acid 8	Citric	40	23.6	0.68	10.86	Irritant	Irritant	Non Corrosive	Corrosive	Not tested
Acid 9	Phosphoric	2.5	11.5	0.91	2.65	Irritant	Irritant	Non Corrosive	Corrosive	Non Corrosive
Acid 10	Phosphoric	9	0	2.25	1.27	Not Classified	Not classified	Non Corrosive	Non-corrosive	Non Corrosive
Acid 29	Phosphoric	19.5	0.5	0.9	6.25	Irritant	Irritant	Non Corrosive	Corrosive	Not tested
Acid 30	Lactic	44	0.14	1.09	8.43	Irritant	Irritant	Non Corrosive	Corrosive	Not tested
Acid 31	Sulphamic Citric	7.8 3.185	5	0.94	3.56	Irritant	Irritant	Non Corrosive	Corrosive	Not tested
Acid 32	Glycolic Citric	1.4 5.005	2	2.09	0.87	Not Classified	Not classified	Non Corrosive	Non-corrosive	Not tested
Acid 34	Citric	10	0	1.7	0.0248	Not Classified	Not classified	Non Corrosive	Non-corrosive	Not tested
Acid 35	Citric Glycolic	6.552 1.4	1.5	2	2.1	Not Classified	Not Classified	Non Corrosive	Corrosive	Not tested
Acid 36	Citric Glycolic	9.555 1.4	2.5	1.7	2.8	Not Classified	Not Classified	Non Corrosive	Corrosive	Not tested
Acid 37	Citric Acrylic	6.37 0.96	11.93	1.6	1.2	Not Classified	Not classified	Non Corrosive	Non-corrosive	Not tested
Acid 38	Citric Acrylic	6.37 0.46	15.9	1.5	1.2	Not Classified	Not classified	Non Corrosive	Non-corrosive	Not tested
Acid 39	Citric Sulphamic	0.455 9.5	3.5	0.5	4.05	Not Classified	Irritant	Non Corrosive	Corrosive	Not tested
Acid 40	Citric	4.55	0.5	1.7	0.9	Not Classified	Not classified	Non Corrosive	Corrosive	Not tested
Acid 41	Sulphamic	10	6.85	0.81	4.34	Irritant	Irritant	Non Corrosive	Corrosive	Non Corrosive
Acid 42	Sulphamic	8	2.34	0.5	2.584	Not Classified	Irritant	Non Corrosive	Corrosive	Not tested
Acid 43	Sulphamic	12.8	1.9	< 1	4.258	Not Classified	Irritant	Non Corrosive	Corrosive	Not tested
Acid 44	Methane Sulphonic Salicylic	0.27 0.1	0.51	1.9	0.106	Not Classified	Not classified	Non Corrosive	Non-corrosive	Not tested

RhE Prediction versus *AISE*

8 of 23 formulas were predicted the same in both the AISE and the RhE methods
15 of 23 formulas were "over predicted" in the RhE method versus the AISE method

Overall Predictions versus Reserve

1.78 – Highest acid reserve which resulted in a non-corrosive in the RhE method
4.34 – Highest acid reserve which resulted in a non-corrosive classification *in vivo*
3/3 formulas with an acid reserve ≤1.78 had concordant results between *in vivo* and RhE (all predicted non-corrosive)
3/4 formulas with an acid reserve >1.78 resulted in a more conservative classification using RhE than *in vivo*. All three formulas with conflicting results were corrosive using RhE and non-corrosive *in vivo*. One formula was corrosive by both methods.

RESULTS SUMMARY

- There was 85% agreement between the AISE Method and *in vivo* data. The under prediction of one formula by the AISE method was attributed to a known limitation of the approach. This formula, (Acid # 4) contained 5% of a cationic surfactant known to be corrosive to skin. Young et. al, noted that the presence of corrosive or irritant substances that are not acidic may result in under prediction and additional testing may be required to confirm a non-corrosive
- There was 57% agreement between the RhE Method and *in vivo* data. In all discordant cases, RhE resulted in a more conservative classification.
- There was 35% agreement between the AISE Method when compared to the *RhE* Method. In all discordant cases, RhE resulted in a more conservative classification.
- There was 100% agreement between the RhE Method and *in vivo* data for formulas tested with an acid reserve ≤1.78 %NaOH/100 mL. For formulas with an acid reserve >1.78 the agreement between the RhE prediction and the *in vivo* data was 25%; the RhE resulted in a more conservative classification 75% of the time.
- The RhE Method predicted all formulas containing greater than 10% total acid to be corrosive. However, no correlation between acid type, surfactant concentration or pH within the extreme range was identified.

Discussion/Recommendation

The use of extreme pH alone to classify an acid-containing product as corrosive to skin can result in over prediction. Historical methods to classify such products include testing on animals, consideration of the pH in conjunction with the acid reserve and use of the EU Conventional Method Calculation. Ethical considerations have caused companies doing business in Europe to rely more heavily on calculation methods in recent years. Current and forthcoming regulatory pressures are driving companies to consider non-animal assays to confirm non-corrosive classification for products with extreme pH. Although a limited number of non-animal assays to assess skin irritation and corrosivity have been validated using a defined set of pure chemicals, the test systems have not been validated using complex mixtures and formulations. Accordingly, future investigations should investigate how synergistic effects of complex mixtures of actives, particularly for extreme pH formulations, may impact the predictions in these non-animal tests.

The data summarized here compared the results of the OECD protocols for EpiDerm™ and EpiSkin™ (RhE Methods) to historical classification methods for chemical mixtures with an extreme acid pH. Although the dataset is limited to 23 products, analyses of these data indicate current RhE protocols are not able to accurately identify non-corrosive products when compared to traditional methods and should not be relied upon to confirm a non-corrosive classification for extreme pH acid mixtures.