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# Apparent limitations of OECD TG 431 for classification of acrylic- and methacrylic-based adhesives

A. Hoerst *, M. Hermann, K.R. Mewes, L. Steinmeyer, M. Buchholzer *

*Henkel AG & Co. KGaA, Henkelstraße 67, 40589 Duesseldorf, Germany*

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ABSTRACT

For years, the strive for *in vitro* methods for toxicological assessment suitable to replace animal studies gained progressive importance. OECD Test Guideline (TG) 431 was implemented in 2004, allowing to circumvent animal testing according to OECD TG 404 while reliably predicting skin corrosion potential of many substances and products. However, non-animal assays often show protocol-dependent limitations, that complicate or even prevent the testing of several groups of substances. In this study, the suitability of the OECD TG 431 for assessment of the skin corrosion potential of known acidic, thus often skin corrosive or irritating acrylic and methacrylic acid-based adhesives and monomers, was investigated. The commercially available Phenion® Open Source Reconstructed Epidermis (OS-REp) model, developed at Henkel & Co. KGaA, was used. The EpiDerm™ prediction model was considered most applicable to the Phenion® OS-REp model. All Proficiency Substances listed in OECD TG 431, amongst them six acids, were correctly classified and subcategorized as Skin Corr. 1 A or 1B/C corrosives. The OS-REp model was shown to be suitable for the assessment of skin corrosion potential in accordance with OECD TG 431. However, our results also indicate that acrylic and methacrylic monomer-based adhesives might fall outside the applicability domain of this guideline.

## 1. Introduction

For each chemical, the inherent hazard potential – covering numerous endpoints – needs to be determined to control risks emerging from these chemicals in consumer products. While *in vivo* tests were without alternative for some time, nowadays various reliable *in vitro* methods can be used, providing comparable results to effects seen in *in vivo* studies or human patch tests (Schöffl et al., 2000). One of the most used OECD test guidelines (OECD TG) for *in vitro* testing of skin corrosion properties is OECD TG 431. Using 3D-reconstructed human epidermis (RhE) models, the irreversible skin corrosive effect, *e.g.* necrotic cell death affecting cells of epidermis following treatment with corrosive substances, is assessed after 3 min and 60 min (OECD, 2019). The main read-out parameter is tissue viability based on the reduction of a soluble, yellow tetrazolium salt (MTT) into a hydrophobic, violet formazan by mitochondrial dehydrogenases of unimpaired cells, that can be detected at 570 nm optical density (OD) (OECD, 2019; Vistica et al., 1991). Furthermore, histological examination after exposure to the test substances for the validation of potential skin corrosive effects is recommended (OECD, 2019). Although OECD TG 431 can be reliably applied for a wide range of chemicals, it also has limitations to its usage. The MTT assay as underlying test method can be impaired by chemicals absorbing light at the same wavelengths as formazan or by chemicals that are able to reduce the tetrazolium salt to formazan due to their autoreductive potential. Both leading to high absorbance values which eventually result in apparent high relative viability data and hence an

*Abbreviations:* AA, Acrylic acid; AC, Acetone; A.I.S.E., International Association for Soaps, Detergents and Maintenance Products; ALI medium, Air-liquid interface culture medium; AT, amitrol; B3, Boron trifluoride dihydrate; BE, Bromoacetic acid; CLP, Classification, labelling & packaging; CU, Cumene hydroperoxide; DA, Dichloroacetyl chloride; EA, Ethanolamine; ECHA, European Chemicals Agency; GCL, Generic concentration limit; GL, Glyoxylic acid monohydrate; HCL, Hydrochloric acid; HE, Hydroxyethyl methacrylate; HP, Hydroxyethyl methacrylate phosphate; IP, Intellectual property; KOH, Potassium hydroxide; LA, Lactic acid; LC, Lauric acid; LDH, Lactate dehydrogenase; MAA, Methacrylic acid; MB, 4-(methylthio)benzaldehyde; MMA, Methyl methacrylate; MTT, 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide / tetrazolium salt; NC, Negative control; OD, Optical density; OECD, Organization for Economic Co-operation and Development; OS-REp model, Open-source reconstructed epidermis model; PB, 2-bromoethylbenzene; PBS, Phosphate buffered saline; PC, Positive control; PH, Phenol; RhE, Reconstructed human epidermis; SCL, Specific concentration limit; SD, Standard deviation; TG, Test guideline.

* Corresponding authors.
*E-mail addresses:* angelika.hoerst@henkel.com (A. Hoerst), miriam.buchholzer@henkel.com (M. Buchholzer).

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under-prediction of the corrosive potential (false-negative classification) (Berridge and Tan, 1993; Chakrabarti et al., 2001; York et al., 1998). Furthermore, substances with pH < 5.5 can lead to a shift of the absorption maximum of formazan to lower wavelength areas and might therefore result in an underestimation of viability and an over-prediction of skin corrosive effects (Plumb et al., 1989). One substance group partly consisting of highly acidic chemicals are industrial adhesives. Methyl methacrylate adhesives, which are mostly used as two component adhesives, are of particularly high importance. These consist of a resin component (Part B) and a hardener component (Part A), which polymerize when the two parts are combined. The reactive part and hence the part of interest when it comes to toxicity testing is the hardener component (Part A), containing the reactive acrylate monomers (REACH Dossier, n.d.).

In Europe, the classification of mixtures, including adhesives, is dependent on its single ingredients and their respective classifications as described in the "Guidance on the Application of the CLP Criteria" provided by the European Chemicals Agency (ECHA) (European Chemicals Agency, 2017). Skin corrosion is considered an additive effect, thus the classification of products is mainly determined using the summation method according to the Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures. Mixtures containing ≥5% of one or more skin corrosive substances are considered to be skin corrosive as well, provided that the generic concentration limit (GCL) applies for the respective substances (Regulation (EC), 2008). The category is then determined by the sum of the highest classified categories.

For some substances specific concentration limits (SCLs) apply (European Chemicals Agency, 2017). These can be higher or lower compared to the GCL, dependent on the substance specific potency to elicit the respective effect. The criteria for the classification of mixtures according to the summation approach are shown in Table 1.

The summation method only provides theoretical classifications, since synergistic or antagonistic effects are not considered. In addition, in the absence of any other data, pH-extreme products with a pH ≤ 2.0 or ≥ 11.5 have to be considered to be corrosive to skin and have to be classified as corrosive to skin unless the alkaline or acid reserve suggests that the product may not be corrosive (Young et al., 1988). This needs to be confirmed by other data, preferably by data from an appropriate validated *in vitro* test. For both, products containing ingredients with extreme pH, as well as products for which synergistic or antagonistic effects cannot be ruled out, an *in vitro* test would be required to assess the actual skin corrosion potential, thereby circumventing *in vivo* testing according to OECD TG 404 (OECD, 2015a). In this study, the skin corrosion potential of adhesive chemicals and products was examined according to OECD TG 431. Currently five validated test methods with commercially available RhE models can be used to assess skin corrosive properties – EpiSkin™ Standard Model, EpiDerm™ Skin Corrosivity Test (EPI-200), SkinEthic™ RHE1, epiCS® and LabCyte EPI-MODEL24 (OECD, 2019). Diverse protocols, validation criteria and classification limits apply for the individual RhE models. Information on reliability, relevance (accuracy) and limitations, mainly in respect of the applicability domain of the RhE models are provided. Prior to routine use of any of the five validated RhE test methods, laboratories should demonstrate technical proficiency by correctly classifying the twelve Proficiency Substances from different substance classes covering all classification categories (OECD, 2019) and only models with comparable proficiency might be used for the assessment. In the present study, the Phenion® OS-REp model, described by Mewes and Groeber (Mewes et al., 2016; Groeber et al., 2016) was used for the assessment of the skin corrosion potential of adhesive ingredients and products according to OECD TG 431. In contrast to the already validated skin models, the production protocol for this model is publicly available (Mewes et al., 2016; Groeber et al., 2016). Thus, the Phenion® OS-REp model could be produced and applied at the Henkel laboratories in high numbers needed for the experiments, which enabled skin corrosion testing independent of commercially available RhE models. As shown by Chacon and OECD colleagues in 2020, open-source models can be equally suitable for skin corrosion testing as the models described in OECD TG 431 and offer the advantage of autonomous testing (Chacón et al., 2020). The OS-REp skin model is already validated according to OECD standards, though not officially accepted for the usage according to OECD TG 439, to determine skin irritation properties (Mewes et al., 2016; Groeber et al., 2016). For the assessment of skin corrosive effects according to OECD TG 431 applying the OS-REp model, the protocol as well as the prediction model for EpiDerm™ skin model was used as a template, as both models show similar diameters of 0.63 $cm^3$ (OECD, 2019; Mewes et al., 2016). Hence, the classification limits shown in Table 2 are applied for the classification of substances tested in this study (OECD, 2019).

**Table 1**
Classification criteria for mixtures according to the summation approach representing the GCL.

| Concentrations of classified ingredients | Mixture classification | |
|---|---|---|
| | Skin Corr. 1 A, B or C | Skin Irrit. 2 |
| Skin Corr. 1 A, B or C | ≥ 5% | ≥ 1% and < 5% |
| Skin Irrit. 2 | | ≥ 10% |

## 2. Material and methods

### 2.1. Adhesive ingredients

In this study, ingredients commonly found in methacrylic and acrylic acid-based adhesives were tested for their skin corrosion potential - hydroxyethyl methacrylate phosphate (HP; CAS: 52628–03-2 Sigma-Aldrich®), acrylic acid (AA; CAS: 79–10-7, Sigma-Aldrich®), cumene hydroperoxide (CU; CAS: 80–15-9, Merck), methacrylic acid (MAA; CAS: 79–41-4, Merck), methyl methacrylate (MMA; CAS: 80–62-6, Sigma-Aldrich®) and hydroxyethyl methacrylate (HE; CAS: 868–77-9, Merck). Classifications of the respective ingredients can vary depending on the data source (CLP, Annex VI; REACH registration dossier). However, MMA and HE are consistently described to be non-corrosive but skin irritant substances, whereas for HP, AA, CU and MAA skin corrosion potential was allocated (Table 3) (REACH Dossier, n.d.; Regulation (EC), 2008; REACH Dossier, 2023a; REACH Dossier, 2023b; REACH Dossier, 2023c; REACH Dossier, 2023d; REACH Dossier, 2023e). Corrosive classification under CLP is applicable to the neat chemical substance with a GCL of ≥5% for mixtures. For CU a SCL of ≥10% for classification as Skin Corr. 1B and of ≥3% - < 10% for classification as skin irritant was assigned in CLP, Annex VI (Regulation (EC), 2008). Identical SCLs were maintained in the REACH dossier for CU (REACH Dossier, 2023a). For MAA the SCL of ≥10% for Skin Corr. 1 A and of ≥3% - < 10% for Skin Irrit. 2 classification was only derived in the REACH dossier (REACH Dossier, n.d.). In general, SCLs take precedence over the GCLs in case they are subject to a harmonized classification in Annex VI (European Chemicals Agency, 2017; Regulation (EC), 2008). However, MAA has a regulatory binding classification in Annex VI of CLP without SCL, therefore the SCL is not applied for product classification.

**Table 2**
Prediction model for EU GHS classification depending on tissue viability for the EpiDerm™ protocol according to OECD TG 431.

| Tissue viability | Resulting classification |
|---|---|
| < 50% after 3 min | Corrosive |
| ≥ 50% after 3 min AND < 15% after 60 min | Corrosive |
| ≥ 50% after 3 min AND ≥ 15% after 60 min | Non-corrosive |
| < 25% after 3 min | Optional Subcategory 1 A* |
| ≥ 25% after 3 min | A combination of optional Subcategories 1B and 1C |

**Table 3**
Classification of adhesive ingredients according to CLP and respective registration dossiers.

| Substance | HP | AA | CU | MAA | MMA | HE |
|---|---|---|---|---|---|---|
| Classification according to CLP, Annex VI | – | Skin Corr. 1 A | Skin Corr. 1B (SCL 10%) | Skin Corr. 1 A | Skin Irrit. 2 | Skin Irrit. 2 |
| Classification according to REACH dossier | Skin Corr. 1B | Skin Corr. 1 A | Skin Corr. 1B (SCL 10%) | Skin Corr. 1 A (SCL 10%) | Skin Irrit. 2 | Skin Irrit. 2 |

### 2.2. Adhesive products

Several adhesive products (formulas: Henkel IP) were tested for their skin corrosion potential. Part A of the two component adhesives contains MMA and corrosive material, whereby MAA accounts for the largest share of corrosive ingredients. The MMA content as well as the sum of contained corrosive ingredients in Part A is shown for the different products in Table 4.

### 2.3. pH measurement

For adhesive chemical dilutions, 10% of the respective adhesive chemical was first solved in acetone (CAS 67–64-1, Merck) and afterwards diluted with water, resulting in overall dilutions of 10% adhesive chemical, 60% acetone and 30% water. The pH was measured with a solvent-stable pH electrode and the Software Tiamo 2.5 (Metrohm).

### 2.4. Absorption spectra

Adhesive chemicals in different concentrations (100 µl) dissolved in acetone were mixed with isopropanolic formazan solution (100 µl, 0.05 mg/ml) in a 96-well plate (CAS 67–63-0, J.T. Baker Avantor Performance Materials; CAS: 57360–697, Sigma-Aldrich®). A 1:1 mixture of the formazan solution and acetone served as a negative control. Absorption spectra were measured with a spectrophotometer from 450 nm – 650 nm.

### 2.5. MTT auto-reduction

Undiluted adhesive chemicals (30 µl) were mixed with MTT (CAS 298–93-1, Sigma-Aldrich®) solution (1 mg/ml; 300 µl) in isopropanol in a 24-well plate without cells. Absorption was measured at 570 nm after 3 min, 30 min, 60 min, 120 min and 180 min incubation.

### 2.6. OS-REp skin model

The Phenion® OS-REp models were produced as described by Mewes et al. (Mewes et al., 2007).

**Table 4**
Adhesive product compositions.

| Product name | MMA Content in Part A [%] | Sum of corrosive ingredients in Part A [%] |
|---|---|---|
| A | 54.60 | 5.11 |
| B | 42.85 | 6.04 |
| C | 54.42 | 5.11 |
| D | 53.85 | 9.46 |
| E | 43.85 | 11.30 |
| F | 44.84 | 7.99 |
| G | 40.55 | 9.66 |
| H | 27.44 | 12.77 |
| I | 40.72 | 7.19 |
| J | 56.69 | 6.86 |

### 2.7. Skin corrosion testing

For skin corrosion testing with the OS-REp skin model, the test protocol for the EpiDerm™ skin model according to OECD TG 431 was used with minor modifications (OECD, 2019). The tissues were treated on Day 19 of air-liquid interface culture for skin corrosion testing. For the application of adhesive products, a positive displacement pipette was used. For the testing of adhesive chemicals, the washing step deviated from the protocol. As the chemicals could not be completely removed by washing with PBS as described in the protocol, the applied substance was gently aspirated with a sterile pipette after the incubation. Afterwards the culture inserts were dipped twenty times into a beaker with PBS to prevent a detachment of a potentially damaged epidermis by rinsing. After washing, the inserts were transferred into a 24-well plate with 200 µl ALI medium each until all models were washed, to prevent them from drying up. For the testing of adhesive products, the hardened product was removed mechanically from the epidermis with sterile tweezers, thereby taking care not to damage the tissue surface, followed by ten rinsing steps with each 600 µl PBS using a multidispenser pipette. Distilled water was used as a negative control. Glacial acetic acid (CAS 64–19-7, Merck) served as a positive control, as KOH (CAS 1310-58-3, Roth) led to the detachment of the epidermis from the tissue model. Empty wells served as blank. The MTT assay was performed as described in the EpiDerm™ protocol (DB-ALM Protocol No. 119) (OECD, 2019; *DB-ALM Protocol no 119: EpiDermTM Skin Corrosivity Test, ECVAM DB-ALM: Protocol [Internet]*, 2023).

### 2.8. LDH measurement

OS-REp skin models were cultivated in phenol red-free ALI medium (Mewes et al., 2007) for 2 days, before the tissue models were treated with the adhesive compounds (50 µl) for 3 min or 60 min. The culture medium was stored at 4 °C overnight. To determine the total LDH released from a tissue, three OS-REp tissue models were preincubated with Triton-X-100 solution (CAS 9002-93-1, Sigma-Aldrich®) in PBS (1%, *w/v*; 5.5 ml) for 24 h and the respective culture medium was used as a pooled positive control for overall amount of release LDH. For the LDH measurement, conditioned culture medium (100 µl) was given into each three wells of a 96-well plate. The LDH mix (Cat. Nr. 11,644,793,001, Sigma-Aldrich®) was prepared in a 1:46 ratio and 100 µl were given to the test samples. The absorption was measured immediately according to the instructions of Roche, recording changes in absorption over 20 min at two-minute intervals.

### 2.9. Histological assessment

For the assessment of adhesive ingredients, the OS-REp models were treated with each chemical (10%, *v/v*) for 30 min. For the assessment of adhesive products, the OS-REp models were treated as described in "LDH measurement". All tissue models were washed with PBS and immediately cryo-preserved in TissuTek® Cryomold® (Sakura) in tissue freezing medium (Leica) at −80 °C. After equilibration to - 20 °C for approximately 1 h, the skin models were cut into 8 µm sections with a Cryomicrotome (Cryo Star NX70, Thermo Fisher Scientific). After sectioning, the tissue slides were stained with hematoxylin/eosin.

## 3. Results

In this study, the ingredients HP, AA, MAA, CU, HE and MMA of methacrylic and acrylic acid-based adhesives as well as adhesive products were tested for their skin corrosion potential. As the physicochemical properties of the single adhesive ingredients, such as low solubility in water and high acidity, can interfere with the commonly used MTT assay read-out described in OECD TG 431 (OECD, 2019; Plumb

et al., 1989), *in vitro* testing of these substances can be hampered. Hence, the suitability for skin corrosion testing of adhesive ingredients and products with the OS-REp model was assessed.

### 3.1. Physical and chemical characterization of adhesive ingredients

#### 3.1.1. pH measurement

Since acidic substances might influence the MTT assay (Plumb et al., 1989) and thereby the read-out of skin corrosion testing according to OECD TG 431, the pH of all adhesive chemicals was initially measured (Table 5). Overall, MMA, HE and CU appeared to be weak acidic chemicals, whereas MAA, AA and HP were shown to be strongly acidic. The acidity of the tested adhesive chemicals ranged from pH values of 5.99 for weak acidic chemicals such as MMA to 0.96 for substantially acidic chemicals such as HP.

#### 3.1.2. pH-dependent absorbance shift

In 1989, Plumb et al. have demonstrated that a spectrum of formazan solution in DMSO at pH 3–5 has lower absorbance than other spectra at higher pH. It has also been shown that a second peak besides 570 nm occurs at 510 nm (Plumb et al., 1989) at low pH. Measurement only at 570 nm would lead to an underestimation of the produced formazan and thus the viability of the cells. Therefore, the influence of the pH of the adhesive chemicals (Table 5) on the absorption peak for formazan was evaluated. Absorption spectra between 450 nm and 650 nm were measured representatively for several concentrations of one weakly acidic acrylic acid derivative (A: HE, pH 4.34) and one highly acidic acrylic acid derivative (B: HP, pH 0.96) (Fig. 1).

Whereas no shift in absorption was detected for weakly acidic HE (Fig. 1A), a significant decrease of formazan absorption as well as the distinct shift of the peak into the short-waved area was observed for the highly acidic HP after the treatment with 50% and 25% solutions (Fig. 1B).

#### 3.1.3. Auto-reductive potential

Moreover, the chemicals were tested for their auto-reductive potential against MTT. The tetrazolium salt solution was mixed with isopropanol to prevent the crystallization and deposit of generated formazan. Undiluted chemicals were added and the OD was measured at 570 nm after 3, 30, 60, 120 and 180 min, respectively (Fig. 2). Besides the positive control, none of the tested undiluted adhesive chemicals led to MTT reduction to formazan and increased OD at 570 nm at any tested timepoint.

### 3.2. Applicability evaluation of the OS-REp model for OECD TG 431

For the evaluation of the OS-REp model for the testing according to OECD TG 431, the classification of twelve Proficiency Substances of diverse substance classes was assessed using a slighty modified protocol initially developed for the EpiDerm™ model (OECD, 2019). The results were compared to the established CLP classification of the substances based on *in vivo* studies. The MTT assay was performed, and the OD was measured at 570 nm after 3 min and 60 min incubation with the respective substance (Fig. 3).

**Table 5**
pH of 10% solutions of the adhesive chemicals. Adhesive chemicals were diluted in an acetone:water mixture. The pH measurement was performed with a solvent stable electrode and the software Tiamo 2.5.

| Chemical (10% solution) | Measured pH |
|---|---|
| HP | 0.96 |
| AA | 2.58 |
| MAA | 2.96 |
| CU | 4.29 |
| HE | 4.34 |
| MMA | 5.99 |

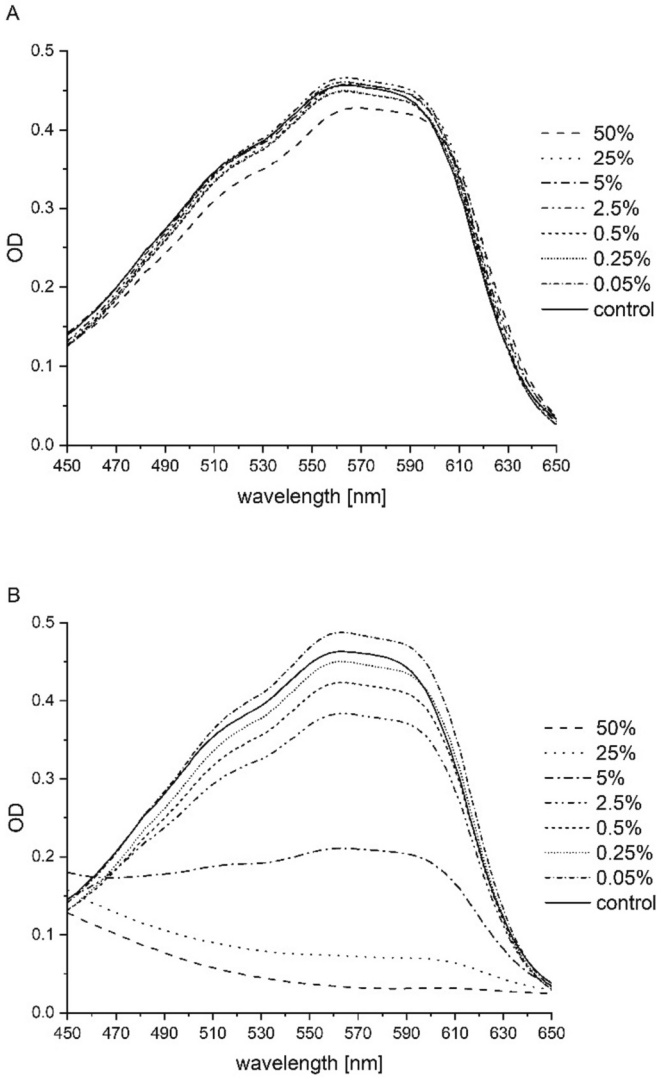


**Fig. 1.** Absorption spectra of formazan after adding different concentrations of adhesive chemicals. Test chemical diluted in acetone (100 µl) was mixed with isopropanolic formazan solution (0.05 mg/ml; 100 µl). The graphs show the absorption spectrum of formazan at wavelengths of 450 nm to 650 nm for representative adhesive ingredients. The mean absorption of formazan from three measurements each is shown after adding solutions of different concentrations (0.05% - 50%) of (A) hydroxyethyl methacrylate (HE) (B) or hydroxyethyl methacrylate phosphate (HP). A 1:1 mix of the isopropanolic formazan solution and acetone was used as a control. ($n$ = 3, OD = optical density).

The results gained for the Proficiency Substances led to correctly predicted classifications using the prediction model described in Table 2. For BE, B3, PH and DA, that are classified as Skin Corr. 1 A, viability <25% was measured after 3 min and < 15% after 60 min incubation. For Skin Corr. 1B/C classified substances, viabilities were > 50% after 3 min and < 15% after 60 min incubation (GL, HCL, LA, EA). For non-corrosive substances, viabilities >50% were observed after 3 min and > 15% were detected after 60 min incubation (PB, LC, MB, AT). In Table 6, the results from this study were compared with the relative viability data which have been published for the EpiDerm™ skin model.

Generally, lower viabilities were measured with the OS-REp model, especially after the treatment with skin corrosive substances. However, the respective classifications were correctly assigned for all substances.

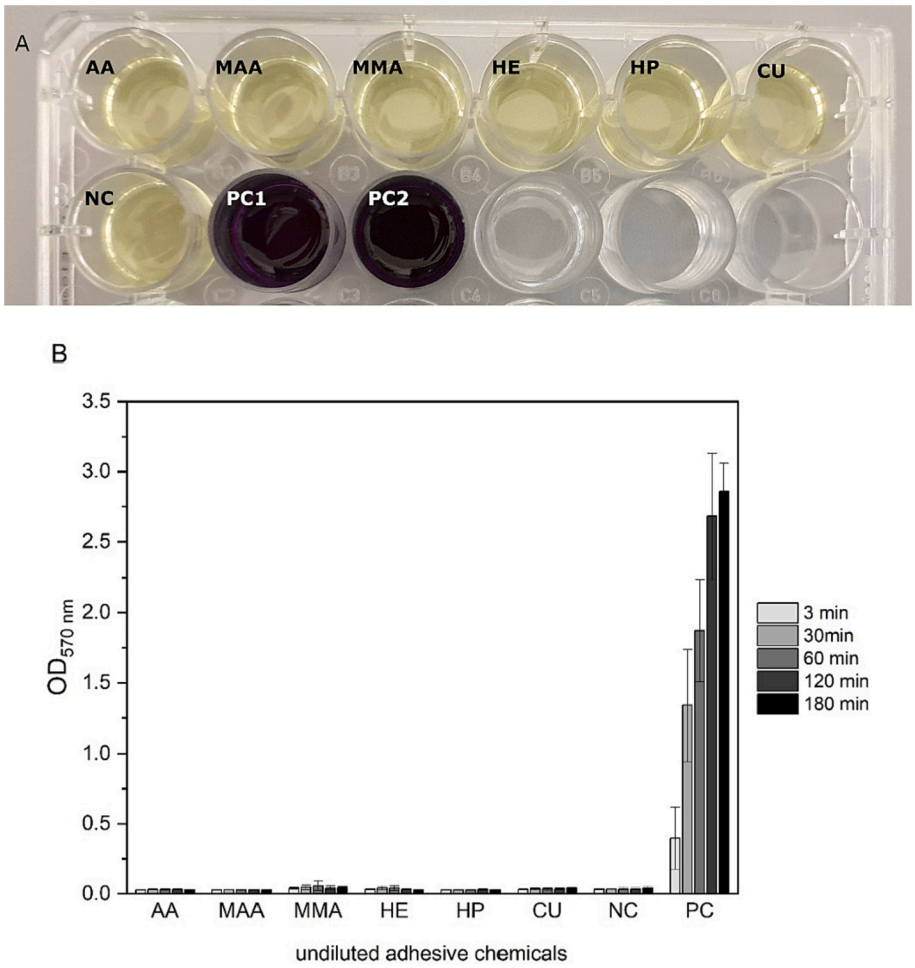


**Fig. 2.** MTT auto-reduction potential of adhesive chemicals. Each undiluted test chemical (30 µl) was added to MTT solution in isopropanol (1 mg/ml; 300 µl). The mixture was incubated at 37 °C. (A) Color of mixtures after 3 h incubation with the respective undiluted chemical. Acetone was used as a negative control (NC), ascorbic acid as a positive control (PC). (PC1 = 10% ascorbic acid solution, 30 µl; PC2 = solid ascorbic acid, undiluted). (B) After 3 min, 30 min, 60 min, 120 min and 180 min incubation with the undiluted test substance, optical density (OD) was measured at 570 nm. Acetone was used as NC, solid ascorbic acid was used as PC. Results are composed of three technical replicates. Error bars show the standard deviation in both directions (±SD, $n = 3$).

### *3.3. Skin corrosion testing of adhesive ingredients with the OS-REp model according to OECD TG 431*

After it was successfully demonstrated that the OS-REp model is suitable to correctly discriminate chemicals based on their skin-corrosive potential, the tissue model was used to test adhesive ingredients concerning their skin corrosion properties according to OECD TG 431. HP, AA and CU were tested in dilutions of 10%, 5% and 2% due to their typical percentages in structural adhesives and also covering the GCL for corrosivity as mean concentration. MAA, that is commonly used in higher concentrations in adhesive products, was tested at concentrations of 20%, 10% and 5%. This concentration range covers the SCL of ≥10% MAA based on the REACH dossier in order to confirm that this limit is also non-corrosive *in vitro*.

HE and MMA, both classified as Skin Irrit. 2 under CLP Regulation (Regulation (EC), 2008), were tested undiluted. For MMA additionally 50% and 25% concentrations were tested to represent typical concentrations in adhesive products. The MTT assay was performed and the OD was measured each at 570 nm after 3 min and 60 min (Fig. 4) with the adhesive ingredients.

For HP, AA and CU, viabilities >50% were detected after 3 min incubation with 2% or 5% dilutions. The same was observed for 10% dilutions of HP and CU. The incubation with the highest concentration of 10% AA for 3 min led to an explicitly decreased viability of <15%. With 5% MAA, a tissue viability of clearly >25% was observed after 3 min, while the higher concentrations of 10% and 20% MAA led to distinctly decreased tissue viabilities to <15%. After 60 min incubation with all tested substances in all tested concentrations, diminished viabilities of <15% were measured. The only exception was a viability of >15% for tissues treated with 2% HP.

The tissue viabilities after treatment with the Skin Irrit. 2 classified substances HE and MMA after 3 min incubation were > 50% at any concentrations. Comparable high tissue viabilities were observed for 100% HE and 25% MMA after 60 min. Testing of 50% MMA resulted in decreased viabilities <50%, whereas 100% MMA reduced the viability even to <15% after 60 min. The viability data determined with the OS-REp model would result in the classifications depicted in Table 7.

Besides the tissue viability check using the MTT assay, a histopathological examination of the treated skin model was performed. The OS-REp model was incubated for 30 min with 10% dilutions of representative adhesive chemicals (AA, CU) or the neat substance (MMA). Treated cryosections of the OS-REp models, were compared to a sham-treated negative control. Representative histological examinations of Hematoxylin/Eosin-stained models are shown in Fig. 5.

While the untreated skin model shows evenly distributed cells with tight cell-cell connections with nucleus-free pink *stratum corneum* and violet basal cell layer, diverse histological changes were observed after treatment with adhesive ingredients. After incubation with AA, the

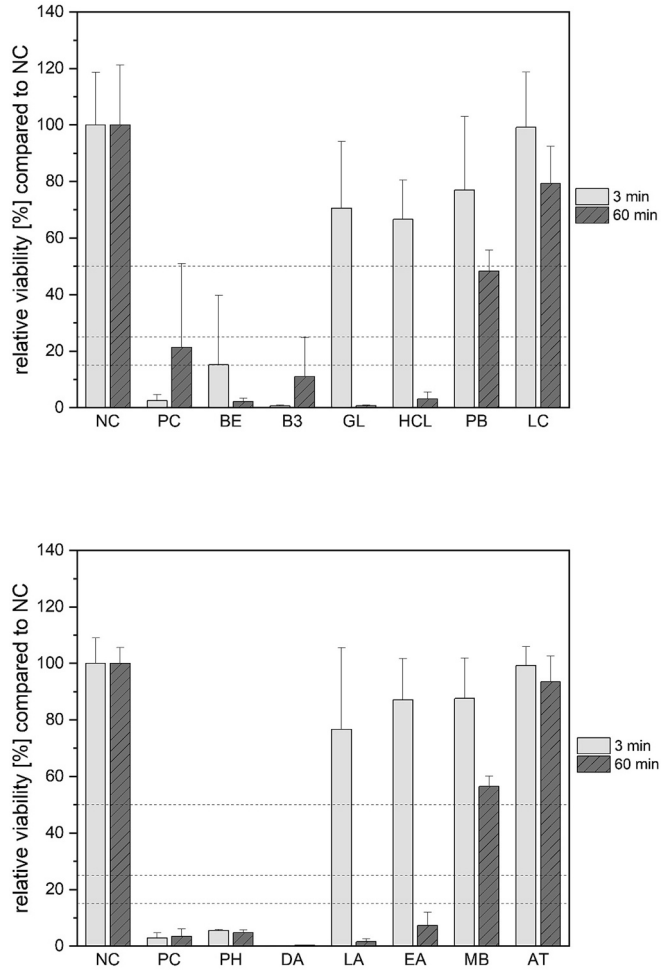


**Fig. 3.** Tissue viability after 3 min and 60 min treatment with Proficiency Substances. The OS-Rep models were treated with liquid Proficiency Substances (50 µl) or solid Proficiency Substances (25 mg/25 µl distilled water) and were incubated for 3 min (light grey) and 60 min (dark grey, hatched). The OD of the isopropanol eluate was measured at 570 nm and the graphs represent the relative tissue viability of treated tissues normalized to negative control (NC). Three OS-REp models were used for each treatment, distilled water was used as NC and glacial acetic acid as positive control (PC). Dashed lines at 50%, 25% and 15% depict tissue viability limits for classification as described in Table 2. (BE = bromoacetic acid, B3 = boron trifluoride dihydrate, GL = glyoxylic acid monohydrate, HCL = hydrochloric acid, PB = 2-bromoethylbenzene, LC = lauric acid, PH = phenol, DA = dichloroacetyl chloride, LA = lactic acid, EA = ethanolamine, MB = 4-(methylthio)benzaldehyde, AT = amitrol). + SD.

*stratum corneum* showed compromised structures, pointing to a breakdown of cell adhesion molecules. The *stratum spinosum* was moreover heavily damaged, lacking its characteristic morphology. In contrast to the loosened-up structure after AA treatment, the treatment with CU led to a highly condensed appearance of the skin tissue up to one third compared to the untreated model. Characteristic cell structures of the single layers were barely perceived. Solely treatment with MMA did not show substantial changes compared to the untreated skin model. All cell layers were clearly recognizable.

### *3.4. Skin corrosion testing of adhesive products with the OS-REp model according to OECD TG 431*

In the next step, also products containing the previously tested adhesive ingredients should be investigated with the OS-REp skin model according to OECD TG 431 and the protocol for EpiDerm™. The MMA content and sum of corrosive ingredients contained in the respective products are shown in Table 4. The skin corrosion test was performed for 3 min and 60 min (Fig. 6).

**Table 6**
Comparison of evaluation data of the OS-REp model and validation data of the EpiDerm™ model as described in OECD TG 431. Data for mean cell viability evaluated with EpiDerm™ were taken from Table 1 of the OECD TG 431. The mean cell viability was determined by using three tissue models per chemical. The formazan measurement was performed with two technical replicates from each model (mean ± SD).

| Substance | Mean cell viability EpiDerm™ [%] | | Mean cell viability OS-REp [%] | | Classification according to GHS |
|---|---|---|---|---|---|
| Incubation | 3 min | 60 min | 3 min | 60 min | |
| Bromoacetic acid (BE) | 3.2 | 2.8 | 1.1 ± 1.2 | 2.7 ± 0.1 | Skin Corr. 1A |
| Boron trifluoride dihydrate (B3) | 4.4 | 10.1 | 0.9 ± 0.1 | 3.4 ± 0.2 | Skin Corr. 1A |
| Phenol (PH) | 22.6 | 13.5 | 5.2 ± 0.2 | 5.2 ± 1.1 | Skin Corr. 1A |
| Dichloroacetyl chloride (DA) | 1.3 | 1.4 | 0.2 ± 0 | 0.3 ± 0 | Skin Corr. 1A |
| Glyoxylic acid monohydrate (GL) | 90.4 | 3.1 | 47.9 ± 4.4 | 0.4 ± 0 | Skin Corr. 1B/C |
| Hydrochloric acid (HCL) | 80.8 | 9.0 | 54.7 ± 6.1 | 4.2 ± 0.2 | Skin Corr. 1B/C |
| Lactic acid (LA) | 90 | 3.5 | 73.8 ± 0.6 | 2.7 ± 0.6 | Skin Corr. 1B/C |
| Ethanolamine (EA) | 69.7 | 9.3 | 85.1 ± 1.7 | 4.5 ± 0.5 | Skin Corr. 1B/C |
| 2-bromoethylbenzene (PB) | 112.5 | 71.2 | 78.3 ± 2.5 | 37.6 ± 3.3 | Not classified |
| Lauric acid (LA) | 90.7 | 64.4 | 97.0 ± 6.4 | 64.0 ± 2.9 | Not classified |
| 4-(methylthio) benzaldehyde (MB) | 85.4 | 81.6 | 93.8 ± 3.6 | 56.4 ± 4.1 | Not classified |
| Amitrole (AT) | 105.7 | 88.2 | 97.8 ± 2.3 | 94.8 ± 2.6 | Not classified |

Table 8 shows the resulting classifications according to the EpiDerm™ prediction model (Table 2).

For the products A-F the viability was decreased already after 3 min treatment to <25%. After 60 min, viability was diminished to <15% of the negative control. Also the treatment with the adhesive products G and H resulted in decreased viability to <50% after 3 min. After 60 min, H-treated tissues showed a viability of <15%. For G-treated tissues, a borderline viability of >15% after 60 min incubation compared to the control was observed, if the standard deviation is considered. Only for the treatment with adhesive products I and J, viabilities >50% were observed after 3 min incubation. However, after 60 min, also for these products the viability was diminished to <15%.

According to the results shown in Fig. 6, the products A-F would be classified as Skin Corr. 1 A (Table 8). For the products G-J, the data result in classifications as Skin Corr. 1B/C (Table 8). However, for these products, high standard deviations were observed. The histology of OS-REp models treated with two representative products or the negative control after 3 min and 60 min is shown in Fig. 7.

Product J was chosen as a representative for Skin Corr. 1B/C classified products and product A was chosen as a representative for Skin Corr. 1 A classified products. The untreated OS-REp model showed evenly distributed cells with tight cell-cell connections with nucleus-free pink *stratum corneum* and violet basal cell layer for both timepoints (Fig. 7A1/A2). Whereas the OS-REp model treated with adhesive product J for 3 min (Fig. 7B1) did not show obvious morphological alterations compared to the negative control, the incubation for 60 min resulted in a swollen *stratum corneum* (Fig. 7B2). Further cell layers were not altered. The treatment with product A led to distinct morphological alterations already after the incubation for 3 min (Fig. 7C1). The whole structure was condensed, showing a thinner epidermis as the negative

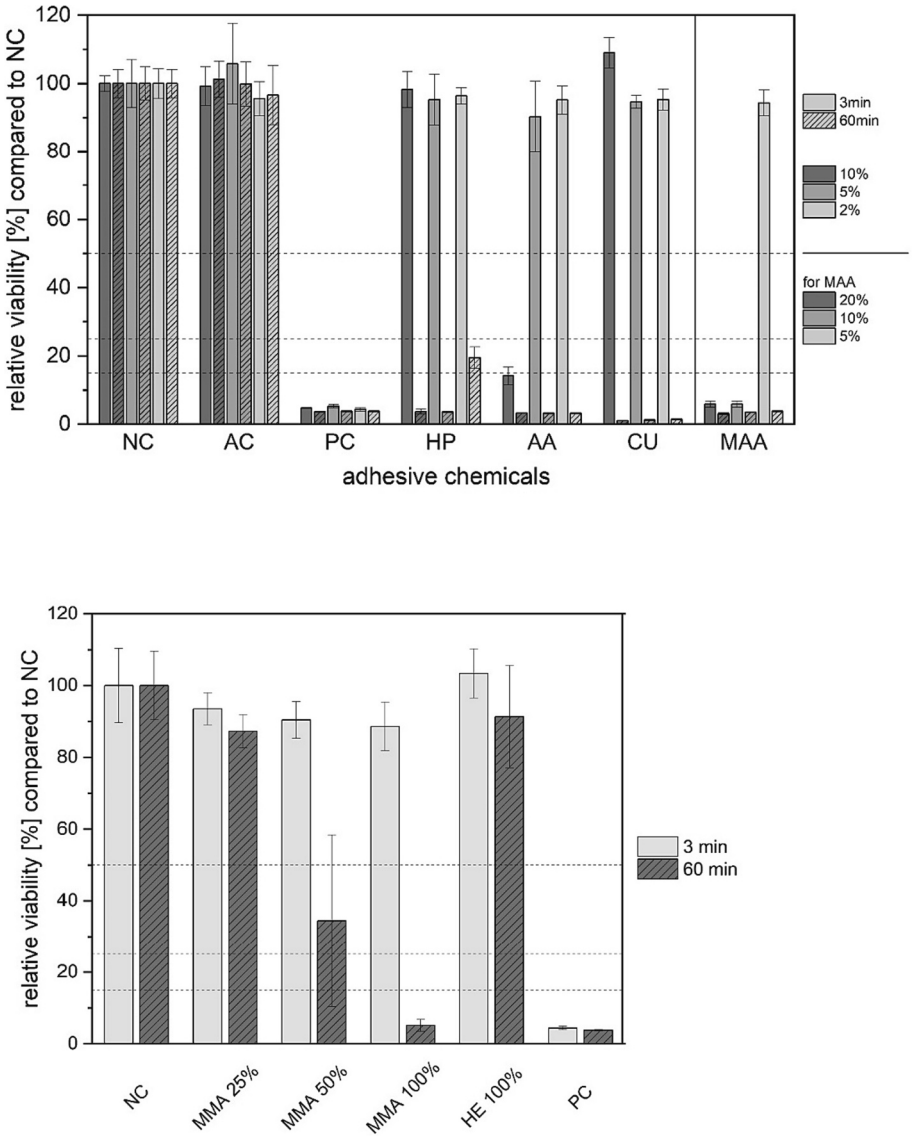


**Fig. 4.** Tissue viability after 3 min and 60 min treatment with adhesive chemicals using the OS-REp model. OS-REp models were treated with 2%, 5% and 10% HP, AA and CU or with 5%, 10% and 20% MAA or with 100% HE or 25%, 50% and 100% MMA for 3 min and 60 min. All chemicals were diluted in acetone. The OD of the isopropanol eluate was measured at 570 nm and the graphs represent the relative tissue viability of treated tissues normalized to negative control (NC). Therefore, several values for the controls are shown, even though there was no dilution for the control tissues. Three OS-REp models were used for each treatment, distilled water was used as a NC, glacial acetic acid as positive control (PC) and acetone as solvent control (AC). Each chemical substance in the respective concentration was tested on one multiwell plate with a NC, PC and AC. The vertical line indicates that bottom legend applies for MAA. Dashed lines at 50%, 25% and 15% depict tissue viability limits for classification as described in Table 2. ± SD.

control. The *stratum corneum* was swollen and comparable to the results for product J after 60 min incubation. After 60 min, the swollen *stratum corneum* was not observed anymore, but the condensation of the *stratum spinosum* to maximum 2 cell layers was clearly detected (Fig. 7C2).

As acidic substances, that are contained in the adhesive products, might have an impact on the MTT measurement (Plumb et al., 1989), the LDH assay was performed as an additional viability test for comparison. The results are shown in Fig. 8 for the representative products A (Skin Corr. 1 A) and G, H and J (Skin Corr. 1B/C) after the treatment for 3 min and 60 min. The positive control was incubated for 24 h.

For none of the products LDH was increased in the culture medium after 3 min. After treatment with the products A, H and J for 60 min, slightly increased LDH levels were observed in the culture medium. The LDH activity did not relate to the strength of the skin corrosive effect determined and depicted in Table 8 based on the results shown in Fig. 6. For product G, higher levels of LDH were measured in the culture medium, however, a high standard deviation applies for this result. None of the increased LDH levels reached levels measured after the treatment with the positive control.

## 4. Discussion

The aim of this study was the *in vitro* testing of adhesive ingredients and products regarding their skin corrosive effects using the OS-REp model, that was developed at the Henkel & Co. KGaA (Mewes et al., 2016; Groeber et al., 2016), and applying the protocol as defined in the OECD TG 431 (OECD, 2019). The potential influence of adhesive ingredients on the test read-out MTT assay was determined. The testing procedure was performed for adhesive ingredients as well as for adhesive products and the results were compared to established classifications based on *in vivo* data or summation approach.

**Table 7**
Classification of adhesive ingredients according to OECD TG 431 using the OS-REp model. Except for HE, all substances were tested in three dilutions. Results highlighted in green correspond to actual classifications according to CLP, Annex VI depicted in Table 3, those highlighted in red differ from the official classification. The percentages on the left give the concentration of the corresponding dilution.

| Substance | HP | AA | CU | MAA | MMA | HE |
|---|---|---|---|---|---|---|
| 2% | Not corrosive | Skin Corr. 1B/C | Skin Corr. 1B/C | | | |
| 5% | Skin Corr. 1B/C | Skin Corr. 1B/C | Skin Corr. 1B/C | Skin Corr. 1B/C | | |
| 10% | Skin Corr. 1B/C | Skin Corr. 1A | Skin Corr. 1B/C | Skin Corr. 1A | | |
| 20% | | | | Skin Corr. 1A | | |
| 25% | | | | | Not corrosive | |
| 50% | | | | | Not corrosive | |
| 100% | | | | | Skin Corr. 1B/C | Not corrosive |

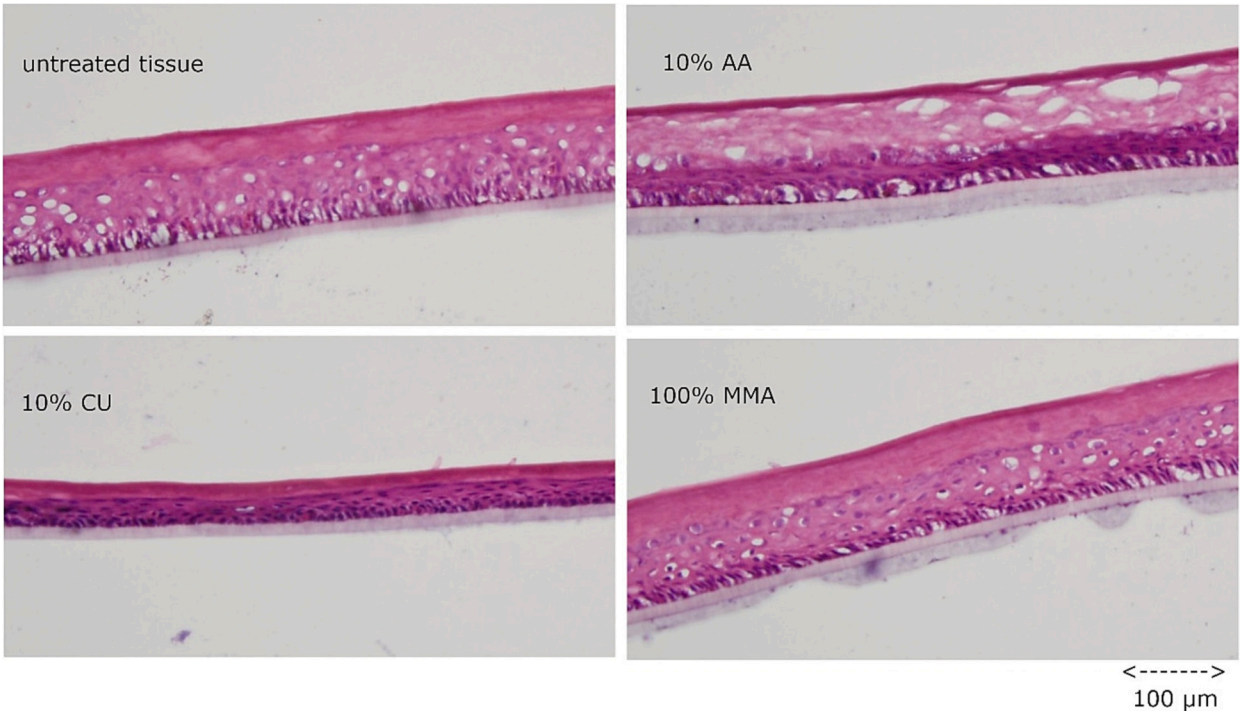


**Fig. 5.** Histological assessment of OS-REp models after treatment with adhesive chemicals. OS-REp models were treated with different concentrations of adhesive ingredients (50 µl) for 30 min. The tissue models were washed with PBS and 8 µm cryosections were prepared. The tissue models were stained with Hematoxylin/Eosin and were compared to the untreated tissue. For all depictions the same scale applies.

### *4.1. The OS-REp model is suitable for skin corrosion testing according to OECD TG 431*

The suitability of the OS-REp skin model for the assessment of the skin corrosion potential of substances according to OECD TG 431 could be shown by the correct classification of Proficiency Substances using a slightly adapted protocol as well as the prediction model for the EpiDerm™ model (Fig. 3, Table 6) (OECD, 2019; Kandarova and Liebsch, 2017). Deviating from the EpiDerm™ protocol, glacial acetic acid was used as a positive control instead of KOH, as the latter led to the detachment of the epidermis from the polycarbonate matrix and its loss during washing. Moreover, the washing procedure was changed to a gentler procedure to prevent the detachment and damage of the epidermis. Defined exposure to the skin model was achieved and standard deviations were found to be in an acceptable range. The viability of OS-REp models treated with the Proficiency Substances was slightly lower than the viability measured with the EpiDerm™ model but led to the same correct classification of the Proficiency Substances as shown in Table 6. Thereby, the OS-REp model qualifies for skin corrosion testing according to OECD TG 431 (OECD, 2019).

### *4.2. Physical and chemical characterization of adhesive ingredients*

Hence, several adhesive ingredients - acrylate derivatives, as well as CU as a polymerization starter - were tested for their skin corrosion potential using the OS-REp model. A 1:3:6 dilution of the test substances in distilled water and acetone allowed the evaluation of their physico-chemical properties such as pH value in advance. High acidic substances appeared to shift the maximum absorption level of formazan into the shorter wavelength area and also reduce the height of the

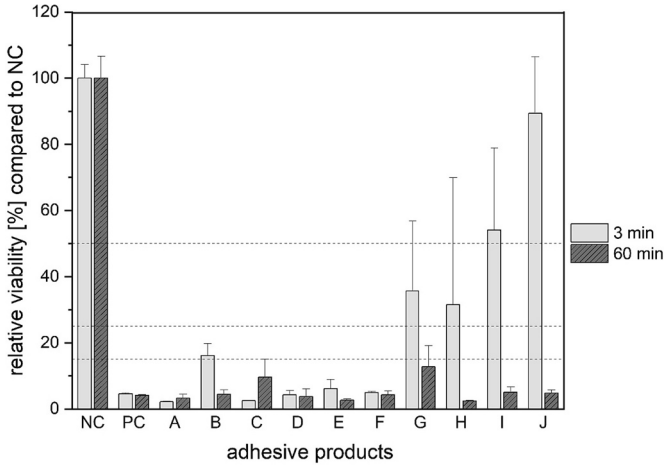


**Fig. 6.** Tissue viability after 3 min and 60 min treatment with adhesive products using the OS-REp model. OS-REp models were treated with the hardener part A of different adhesive products (A-J) for either 3 min (light grey) or 60 min (dark grey, hatched). The adhesive products were mechanically removed and the tissue models were washed according to the modified protocol described in material and methods. The OD of the isopropanol eluate was measured at 570 nm and the graphs represent the relative tissue viability of treated tissues normalized to negative control (NC). Three OS-REp models were used for each treatment. Distilled water was used as NC and glacial acetic acid as positive control (PC). Dashed lines at 50%, 25% and 15% depict tissue viability limits for classification as described in Table 2. + SD.

absorption at 570 nm. This results in discoloration of the dye as it was representatively shown for HP (Fig. 1B) and already described in literature (Plumb et al., 1989). However, an underestimation of cell viability and thereby an overestimation of the skin corrosive effect using the MTT assay is unlikely to occur, considering the tested concentrations. Adhesive ingredients have been diluted several times due to the careful rinsing procedure, resulting in very low amounts of possible residues. Fig. 1 shows that even if lower percentages of acidic residues are present, the influence on the absorption spectra is negligible and cannot serve as an explanation for decrease of MTT formation observed when testing certain acidic adhesive ingredients. Since also an auto-reductive potential of the adhesive chemicals towards MTT was excluded (Fig. 2), no interference of these substances with the MTT assay according to OECD TG 431 was to be expected.

### *4.3. Evaluation of skin corrosion properties of adhesive components according to OECD TG 431*

According to ECHA's "Guidance on the Application of the CLP Criteria", substances and mixtures with extreme pH of ≤2.0 or ≥ 11.5 should be considered as skin corrosive (European Chemicals Agency, 2017). AA with a borderline non-extreme pH (pH 10% 2.58) tested at only 2% resulted in drastically decreased tissue viabilities after 60 min incubation and needs to be classified as Skin Corr. 1B/C. Based on the GCL of 5%, 2% AA would not require classification as corrosive. While 2% AA is over-classified, a concentration of 5% is underestimated as Skin Corr. 1B/C instead of Skin Corr. 1 A, only a concentration of 10% is correctly predicted (Table 7). The same is true when testing MAA (pH 10% 2.96) which was underestimated as Skin Corr. 1B/C when tested at 5% (Table 7). However, MAA is a substance registered under REACH regulation. Registrants have derived a SCL of ≥10% for Skin Corr. 1 A and of ≥3% - <10% for Skin Irrit.2 classification, respectively (REACH Dossier, n.d.). These SCLs are currently not applied under CLP, since MAA has a harmonized mandatory Annex VI classification (Regulation (EC), 2008). The SCL is supported by a short-term dermal repeated dose study in mice. Mice were treated for 3 weeks 3 times a week with aqueous solutions of 4.8%, 9.6% and 19.2% MAA in acetone. Severe irritation was observed with 19.2% MAA. A MAA concentration of 9.6% was considered irritant but not corrosive (REACH Dossier, 2023c). Against this background the GCL of 5% for corrosive classification of MAA according to CLP regulation can be questioned and might be treated as overclassification. HP, the only chemical in the test panel with extreme pH (pH 10% 0.96), was correctly classified as Skin Corr. 1B/C at a concentration of 5% and not classified for skin corrosion at 2% (Fig. 4, Table 7). HP did not affect cell viability after 3 min incubation at any concentration (Fig. 4). Even after 60 min incubation, the lowest concentration of HP showed a comparably lower cytotoxicity. The apparently slightly weaker skin corrosion potential of HP compared to AA and MAA might be explained by a lower buffer capacity and a smaller acidic reserve (Young et al., 1988). A dissociation of HP into phosphoric acid and HE can also be reasonably assumed (EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP), et al., 2020). HE was found to be non-corrosive in this study (Fig. 4, Table 7), furthermore, the SCL for phosphoric acid for classification as Skin Corr. 1B is ≥25% and compared to the commonly applied GCL of ≥5% high (Regulation (EC), 2008). Thus, a dissociation of HP could explain the higher tissue viability after treatment. While the skin corrosion potential of HP was correctly estimated in this study, the skin corrosivity testing of AA and MAA yielded inconsistent results. Testing of CU, which is a free radical initiator for production of acrylates, resulted in correct prediction for 10% CU as Skin Corr. 1B/C and was therefore in line with CLP classification and REACH dossier (Regulation (EC), 2008; REACH Dossier, 2023a), whereas concentrations of 2% and 5% were overpredicted as Skin Corr. 1B/C (Fig. 4, Table 7). Based on the SCLs for CU, concentrations between ≥3% - < 10% would require classification as Skin Irrit. 2 and < 3% even only as non-classified (Regulation (EC), 2008; REACH Dossier, 2023a). Consequently, low amounts of CU used in acrylic and methacrylic adhesives might trigger more severe classifications. Undiluted MMA (pH 10% 5.99), which is only classified as Skin Irrit. 2 according to CLP (REACH Dossier, n.d.; European Chemicals Agency, 2017), would wrongly be classified as Skin Corr. 1B/C. A concentration of 50% MAA reduced the cell viability after 60 min incubation to 40%, although being still considered non-corrosive (Fig. 4, Table 7). The apparent overprediction of skin corrosion properties of MMA by the MTT assay is moreover substantiated by the unaltered histological architecture of MMA-treated tissue models (Fig. 5), that stands in contrast to the drastic reduction of tissue viability shown in Fig. 4. In contrast to MMA, HE was correctly classified as Skin Irrit. 2 (Fig. 4, Table 7).

Altogether, the results suggest, that OECD TG 431 in its current version is unsuitable to reliably classify skin corrosive effects of adhesive chemicals. An overestimation of skin damaging effects for pH-extreme acidic detergents was already described in 2011 (Willems et al., 2016). However, the inconsistent results obtained in this study for highly acidic adhesive ingredients like AA, MAA and less acidic MMA do not indicate any connection between acidity and outcome of OECD TG 431 testing. AA may despite the borderline extreme pH be overpredicted or underpredicted depending on the concentration tested. MMA, that is almost neutral, is heavily overpredicted. Since six of the Proficiency Substances were acidic and were still classified correctly, the acidity of a

**Table 8**
Classification of adhesive products according to OECD TG 431 using the OS-REp model.

| Product | A | B | C | D | E | F | G | H | I | J |
|---|---|---|---|---|---|---|---|---|---|---|
| Classification | Skin Corr. 1 A | Skin Corr. 1 A | Skin Corr. 1 A | Skin Corr. 1 A | Skin Corr. 1 A | Skin Corr. 1 A | Skin Corr. 1B/C | Skin Corr. 1B/C | Skin Corr. 1B/C | Skin Corr. 1B/C |

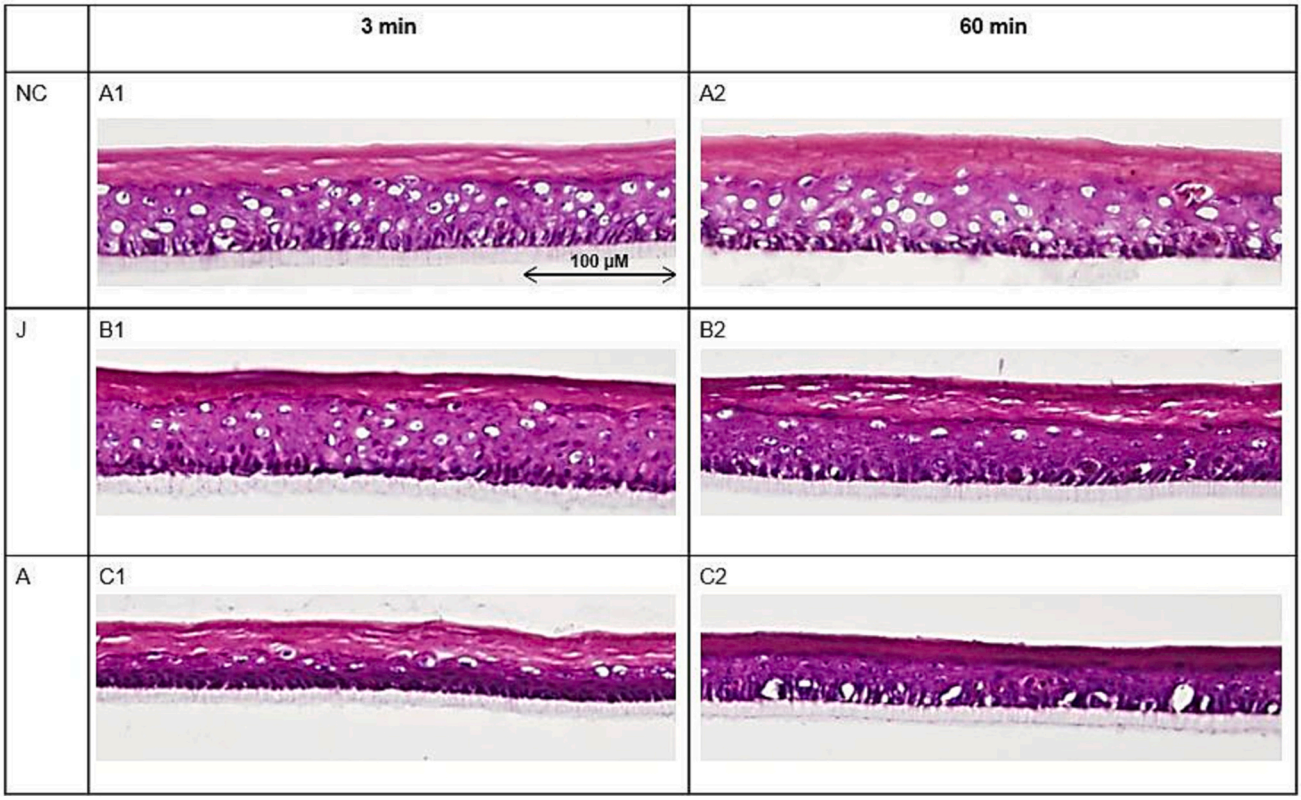


**Fig. 7.** Histological assessment of OS-REp models after treatment with adhesive products. OS-REp model were treated each with product J (B1 & B2) and product A (C1 & C2) (50 µl) for 3 min and 60 min, respectively. The models were stained with Hematoxylin/Eosin and were compared to an untreated tissue as the negative control (A1 & A2). For all depictions, the same scale applies.

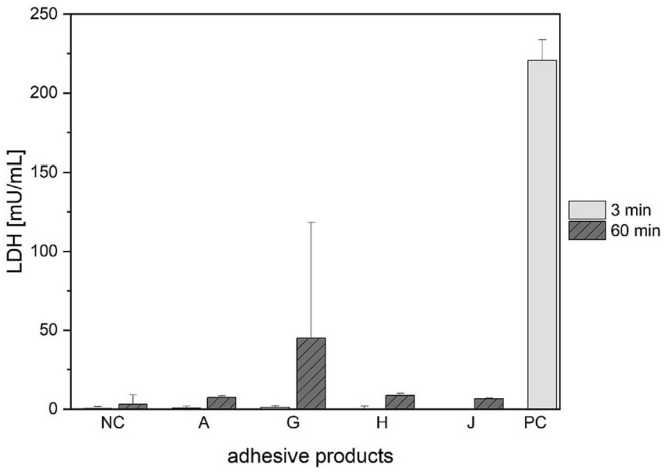


**Fig. 8.** LDH leakage after 3 min and 60 min treatment with adhesive products using the OS-REp model. OS-REp models were treated with the respective adhesive product (50 µl) for either 3 min (light grey) or 60 min (dark grey, hatched). LDH assay was performed according to the manufacturers declarations. Distilled water was used as negative control (NC), Triton-X-100 solution was used as positive control (PC, 1%, 50 µl). The graphs represent the mean LDH activity in milliunits per milliliter of six wells per product, conducted from 2 replicates from one tissue. The data represent one technical experiment. + SD.

substance alone cannot explain why the *in vitro* skin corrosion test failed with acrylic and methacrylic monomers. Although the reason for the observed mispredictions is not yet elucidated, it was clearly demonstrated, that adhesive chemicals such as acrylic and methacrylic monomers are apparently not within the applicability domain of the OECD TG 431.

### *4.4. Evaluation of skin corrosion properties of adhesive products according to OECD TG 431*

The total content of corrosive material in the tested products ranged from 5.11 to 12.77%, whereas the corrosive main constituent in all products was MAA. Three products (A, B, C) comprise only of <6% corrosive ingredients (Table 4), which is mainly MAA. For these three products (Fig. 6) severely decreased cell viabilities of <25% were already detected after 3 min incubation. However, as indicated earlier the GCL of ≥5% for corrosive classification might be too conservative, as animal test data published in the REACH registration dossier of MAA indicate that a SCL of ≥10% might be warranted (REACH Dossier, 2023c). For this reason, classification of products A, B and C as Skin Corr. 1 A may represent an overclassification. In contrast, products G and H with nearly double amount of corrosive ingredients compared to products A, B and C, were slightly better tolerated (Table 4, Fig. 6). Only products I and J showed a mean cell viability >50% after 3 min, but still require corrosive classification due to the low cell viability at the 60 min timepoint (Fig. 6). There is no correlation between the extent of tissue damage and amount of corrosive ingredients. The inconsistency of results obtained with adhesive ingredients can also be observed with adhesive products.

The adhesive ingredient MMA is included in all tested products ≥40%, except for the product H, which contains only 27% of this ingredient. According to the results of the present study, undiluted skin irritant MMA (REACH Dossier, n.d.) was shown to be skin corrosive and would be mistakenly classified as Skin Corr. 1B/C (Fig. 4). Even at a concentration of 50% MMA reduced the cell viability already below 40% after 60 min, which suggests that its presence in adhesives at similar concentrations will contribute to the outcome of corrosivity testing

when using OECD TG 431 (Fig. 4). Furthermore, this suggests that the skin corrosion testing of acrylic and methacrylic-based adhesives according to OECD TG 431 is – despite the successful proficiency testing - overpredictive and therefore not suitable for testing. It was already described, that products with >10% acids generally end up as skin corrosive when tested with RhE models, and that no apparent correlation between acid type or pH within the extreme range was found (Scheel et al., 2011; Willems et al., 2016). However, acidity might not be the only parameter, that impairs the measurement. Besides incorrect classifications also technical issues were faced when testing the skin corrosion potential of adhesive products with the herein described method. The products appeared highly viscous or waxy and an even distribution on the tissue surface was not guaranteed for every tested product. This might be one cause for the high standard errors shown in Fig. 6. As the products are poorly water-soluble and even dried out during the 60 min incubation time, the washing procedure as described for the EpiDerm™ model (OECD, 2019; Kandarova and Liebsch, 2017) were, despite modifications, not sufficient to completely remove all products from the OS-REp model. Even mechanically removal with sterile tweezers was not always sufficient. This might have resulted in prolonged exposure times up to 3 h during MTT incubation especially at the outermost areas of the tissue where chemical removal was hardly possible. This observed uneven distribution of the product on the model, might have additionally resulted in significant deviations between single tissues of the same experimental group and hence to the quite high standard errors (Fig. 6). However, effects due to acidification can be ruled out because no color change of the phenol red containing ALI medium was detected (data not shown). Whereas the MTT assay might have overestimated the corrosive effect of adhesive products, the results from the LDH assay did not point to cytotoxic effects for most tested products. High values in the LDH assay and hence apparently increased LDH leakage was only shown for adhesive product G after 60 min treatment (Fig. 8). However, a large standard deviation was measured, and the comparatively high absorption was rather attributed to a brownish cloudiness of the cell culture medium (not shown). The lack of LDH activity in the cell culture medium indicate that cell plasma membranes were intact and have not been damaged, which is expected for non-corrosive products. However, histological assessment of the same OS-REp models used for LDH activity measurement showed that distinct morphological changes, such as highly compacted tissue layers, were evident after 3 min incubation with, for example, product A (Fig. 7). According to the morphological changes, an increase of LDH in the cell culture medium at least after 60 min would have been assumed. In this case, the histological appearance rather supports the results from the MTT assay performed for this product (Fig. 6, Fig. 7). The reason for this obvious discrepancy might have been a strongly limited enzyme activity of LDH resulting from potentially moderately increased acidity of the cell culture medium, as the maximum enzymatic activity lies at a pH of 8.3 (Gay et al., 1968). The most conclusive explanation for non-increased LDH activity in the cell medium, however, might be the uneven distribution of the tested products on the OS-REp model, that resulted in heterogeneously damaged areas. Whereas the results in Fig. 7 complied with the largest area of the skin model, eventually also contrary unaltered morphology was observed for the same model. A complete covering of the OS-REp surface might have resulted in higher LDH activity. Unaltered histology and an intact integrity of the *stratum spinosum* after 60 min treatment (Fig. 7) however speaks against a skin corrosion potential of product J as predicted by MTT (Fig. 6) and thereby for an overestimation of skin corrosion potential of adhesive products using the OECD TG 431.

## 5. Conclusion

The aim of this study was to evaluate the suitability of the OS-REp model for *in vitro* testing of the skin corrosive effects according to OECD TG 431 as well as the testing of adhesive ingredients and products, to further check its applicability domain. Technical proficiency was demonstrated for all twelve Proficiency Substances listed in OECD TG 431. All substances, amongst them six acids, were correctly classified and subcategorized as Skin corr. 1 A or 1B/C corrosives. However, the results presented in this study suggest that the adhesive ingredients and products might not fall within the applicability domain originally defined for this *in vitro* test method. For the adhesive monomers over- and underpredictions as corrosive or non-corrosive occurred, which cannot be explained by unspecific MTT reduction or pH-dependent decrease of the MTT formazan absorption maximum. All tested adhesives yielded corrosive results. No correlation between the content of corrosive ingredients and the extent of tissue damage was found. In addition, evaluation of adhesive products was hampered by uneven distribution onto the tissue models in some cases and difficulties to properly remove the test substance. Results indicate that LDH determination might not be better suited for assessment of cytotoxicity when compared to the MTT test. Other *in vitro* OECD tests such as the OECD TG 435 (Corrositex®) have to be considered for correct product classification. OECD TG 435 would even offer advantages over OECD TG 431 since it allows determination of all three skin corrosion subcategories (OECD, 2015b). However, the reasons for the mispredictions for adhesive chemicals and products remains still unclear and needs to be further explored.

## Declaration of Competing Interest

The authors declare that there are no competing interests associated with the manuscript.

## Data availability

Data will be made available on request.

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