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Deriving a point of departure for assessing the skin sensitization risk of wearable device constituents with *in vitro* methods

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ABSTRACT

Wearable devices are in contact with the skin for extended periods. As such, the device constituents should be evaluated for their skin sensitization potential, and a Point of Departure (PoD) should be derived to conduct a proper risk assessment. Without historical *in vivo* data, the PoD must be derived with New Approach Methods (NAMs). To accomplish this, regression models trained on LLNA data that use data inputs from OECD-validated *in vitro* tests were used to derive a predicted EC3 value, the LLNA value used to classify skin sensitization potency, for three adhesive monomers (Isobornyl acrylate (IBOA), N, N- Dimethylacrylamide (NNDMA), and Acryloylmorpholine (ACMO) and one dye (Solvent Orange 60 (SO60)). These chemicals can be used as constituents of wearable devices and have been associated with causing allergic contact dermatitis (ACD). Using kinetic DPRA and KeratinoSens™ data, the PoDs obtained with the regression model were 180, 215, 1535, and 8325 μg/cm^2 for IBOA, SO60, ACMO, and NNDMA, respectively. The PoDs derived with the regression model using NAMs data will enable a proper skin sensitization risk assessment without using animals.

1. Introduction

Prevention of allergic contact dermatitis (ACD) remains an important focus area of toxicology. In recent years, significant progress has been made in developing and validating skin sensitization New Approach Methods (NAMs), which are defined as any technology, methodology or approach that can provide information for chemical hazard and risk assessment to decrease the reliance on animal testing (EPA, 2021). These validated NAMs have been adopted by the Organization for Economic Cooperation and Development (OECD) in a series of three key event-based test guidelines (TG), which describe many different test protocols (OECD, 2022b, 2023b, c). In addition, the first OECD guideline implementing three Defined Approaches (DA) No. 497 (OECD, 2023a), which combines data from several OECD TG NAMs to predict skin sensitization potential, was adopted (OECD, 2021). The published DA integrates data derived from two or three OECD TG methods: the Direct Peptide Reactivity Assay (DPRA) (Gerberick et al., 2004), the KeratinoSens™ assay (KS) (Emter et al., 2010), and the human cell line activation test (h-CLAT) (Sakaguchi et al., 2006), and in silico data to address hazard and potency classification. The addition of other test methods is currently under review.

The DA 'Integrated Testing Strategy (ITS)' (Takenouchi et al., 2015) and the kinetic Direct Peptide Reactivity Assay (kDPRA) (Natsch et al., 2020; Wareing et al., 2020) were adopted by the OECD (TG 442C) as approaches to discriminate the Global Harmonized System (GHS) potency classes 1A or 1B/not classified, in where 1A corresponds to a strong sensitizer and 1B to a weak sensitizer. However, these approaches do not generate a point of departure (PoD) for quantitative risk assessment (QRA) (Api et al., 2020; Api et al., 2008). Assessing skin sensitization potency and determining a PoD is required for conducting next-generation risk assessments (NGRA) on new chemical entities for which only non-animal information is available (Api et al., 2020; Bernauer et al., 2021; Dent et al., 2018; Gilmour et al., 2020; Gilmour et al., 2022). Assays including the KeratinoSens™ assay, the human cell line activation test (h-CLAT), and the kDPRA yield continuous quantitative data (*i.e.*, concentration-response data) in addition to the binary or categorical outcome. Continuous data sources have been recommended for deriving a PoD for risk assessment, and several models for integrating such data were proposed (Gilmour et al., 2022; Jaworska et al., 2015;

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Abbreviations

ACD	allergic contact dermatitis
ACMO	Acryloylmorpholine
DA	defined approach
DPRA	direct peptide reactivity assay
GHS	Global Harmonized System
h-CLAT	human cell line activation test
IBOA	Isobornyl acrylate
kDPRA	kinetic direct peptide reactivity assay
KS	KeratinoSens™ assay
LLNA	local lymph node assay
MW	molecular weight
NNDMA	N, N- Dimethylacrylamide
pEC3	Predicted Effective concentration
PoD	point of departure
OECD	Organization for Economic Co-operation and Development
SO60	Solvent Orange 60
TG	test guideline
VP	vapor pressure

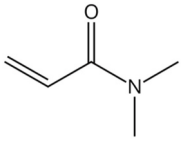
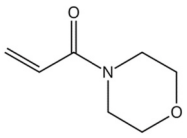
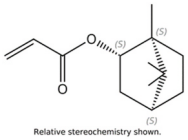
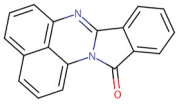
Natsch et al., 2018; Reynolds et al., 2022).

Another quantitative approach has been recently published in which data from OECD-adopted methods were combined in multiple regression models to predict a PoD (Natsch, 2023; Natsch and Gerberick, 2022a, 2022b). The previous work on regression models (Natsch et al., 2015) used kinetic rate constants generated with the Cor1-C420 peptide reactivity assay (Natsch and Gfeller, 2008). The updated linear regression models using OECD-adopted methods (kDPRA, KS, and/or h-CLAT) were built using a comprehensive database of 322 chemicals assembled from previous publications (Natsch et al., 2015, 2020). Comparable to the Bayesian models developed by Jaworska et al., 2013, 2015, the regression models were trained on EC3 values from the Local Lymph Node Assay (LLNA) (*i.e.*, the concentration expected to induce a threshold positive response, *i.e.*, a three-fold increase in cell proliferation in the draining lymph nodes of the treated mice compared to controls).

Evaluating potency information and determining an accurate PoD is critical to ensure any new or existing chemical is safe for exposed workers and consumers. In recent years, there have been numerous case reports of materials used in the manufacturing of wearable products that cause ACD. Solvent Orange 60 (SO60) is a perinone-type dye often used in plastic materials such as spectacle frames, goggles, and gloves that have been shown to cause contact allergy (Ahrensbøll-Friis et al., 2022; Kawakami et al., 2021; Linauskienė et al., 2018; Malinauskiene et al., 2013; Nishihara et al., 2018; Pesonen and Suuronen, 2020; Shono and Kaniwa, 1999; Shono et al., 2018, 2019; Uter et al., 2020; Yeo et al., 2011). Isobornyl acrylate (IBOA) and N, N- Dimethylacrylamide (NNDMA) are monomers used in medical devices such as glucose sensors or insulin pumps and have been reported to cause contact allergic reactions (Gatica-Ortega et al., 2021; Herman et al., 2017, 2018; Hyry et al., 2019; Joyanes Romo et al., 2022; Kamann et al., 2019; Khatsenko et al., 2020; Mowitz et al., 2019; Oppel et al., 2020; Pyl et al., 2020; Svedman et al., 2021; Ulriksdotter et al., 2020; Uter et al., 2020). These adhesive monomers apparently leached from the plastic encasing the device and sensitized patients over prolonged exposure. Acryloylmorpholine (ACMO) was identified in an outbreak of occupational ACD involving workers exposed to adhesives in a smartphone protective case (Gatica-Ortega et al., 2022; Herreros-Montejano et al., 2022). Similarly, ACMO and IBOA have been associated with causing ACD from phone protectors. ACMO is structurally related to NNDMA; however, it is uncertain whether there are cross-reactions between them in practice.

Often, insufficient information is available in the literature to establish skin sensitization induction threshold levels. Our work employed OECD-adopted skin sensitization NAMs to derive a PoD using a linear regression model approach (Natsch and Gerberick, 2022a, 2022b). Specifically, a PoD was determined for four chemicals, including three adhesive monomers (IBOA, NNDMA, and ACMO) and one dye (SO60) associated with ACD. These PoD values help conduct quantitative skin sensitization risk assessments to determine safe exposure levels for workers and consumers (Api et al., 2020; Api et al., 2008).

Table 1
Test chemicals' molecular weight and vapor pressure data.

Chemical Name	CAS RN	SMILES	Structure	MW	Vapor Pressure (mmHg)	Vapor Pressure (Pa)
N, N-Dimethylacrylamide (NNDMA)	2680-03-7	O=C(C=C)N(C)C		99.13	0.821	109.193
4-Acryloylmorpholine (ACMO)	5117-12-4	O=C(C=C)N1CCOCC1		141.17	7.63×10^{-2}	10.15
Isobornyl acrylate (IBOA)	5888-33-5	O=C(OC1CC2CCC1(C)C2(C)C)C=C		208.301	3.87×10^{-2}	5.15
Solvent Orange 60 (SO60)	6925-69-5	O=C1C=2C = CC=CC2C3 = NC4=CC=CC = 5C = CC=C(C45)N13		270.28	1.28×10^{-9}	1.70×10^{-7}

2. Materials and methods

2.1. Test chemicals

Four test chemicals were selected for this investigation: three adhesive monomers and one dye. The adhesive monomers, obtained from Sigma-Aldrich (St. Louis, MO, USA), were N, N-dimethylacrylamide (NNDMA; CAS RN 2680-03-7; 99% purity, contains 500 ppm monomethyl ether hydroquinone as an inhibitor), 4-acryloylmorpholine (ACMO; CAS RN 5117-12-4; 97% purity, contains 1000 ppm monomethyl ether hydroquinone as an inhibitor), and isobornyl acrylate (IBOA; CAS RN 5888-33-5; technical grade >85% purity, contains 200 ppm monomethyl ether hydroquinone as an inhibitor). The dye, Solvent Orange 60 (SO60; CAS RN 6925-69-5; 95% purity), was purchased from AK Scientific, Inc. (Union City, CA, USA). Molecular weights (MW) and vapor pressures (VP) used as input data for the regression models were obtained from the United States Environmental Protection Agency's CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/) (Table 1). Average predicted or experimental VPs were used. VPs were converted from mmHg to Pascals (Pa) by multiplication with a factor of 133.

The assay controls for each of the NAMs, also obtained from Sigma-Aldrich (St. Louis, MO, USA), were Cinnamaldehyde (CAS RN 104-55-2; 99.2% purity) for kDPRA and trans-Cinnamaldehyde (CAS RN 14371-10-9; 99.9% purity) for the Keratinosens™.

2.2. Kinetic Direct Peptide Reactivity Assay (kDPRA)

The kDPRA evaluates the reaction kinetics of a test chemical binding to a synthetic cysteine-containing heptapeptide to derive a maximum rate constant (log k_{max} [Molarity^{-1} seconds^{-1}]), which can be used to classify its skin sensitization potency (*i.e.*, GHS subcategory 1A or GHS subcategory 1B/not classified) (Natsch et al., 2020) or be used in the context of a DA to assess skin sensitization potency (Natsch et al., 2022).

The kDPRA was conducted according to the OECD Test Guideline 442C (OECD, 2023b) and the European Union Reference Laboratory for alternatives to animal testing (EURL ECVAM) DataBase service on Alternative Methods to animal experimentation (DB-ALM) Protocol n° 217: Kinetic Direct Peptide Reactivity Assay (kDPRA). Briefly, a synthetic heptapeptide containing cysteine (Ac-RFAACAA-COOH; RS Synthesis, Louisville KY, USA) was incubated with five concentrations of the test chemical prepared by serial dilution in either acetonitrile or phosphate buffer pH 7.5, typically from a 20 mM stock concentration, for 10, 30, 90, 150, 210, and 1440 min. Twenty mM stock solutions of NNDMA, AMCO, and IBOA were prepared in acetonitrile, resulting in final reaction concentrations of 5, 2.5, 1.25, 0.63, and 0.31 mM. SO60 was not completely soluble in either solvent at 20 mM or 10 mM but was more soluble in acetonitrile than phosphate buffer. Thus, a 5 mM stock solution of SO60 was made with acetonitrile. The resulting final reaction mixture concentrations of SO60 were 1.25, 0.63, 0.31, 0.16, and 0.08 mM.

After each incubation period, the reaction was terminated by the addition of the dye monobromobimane (mBrB; Sigma Aldrich, St. Louis, MO, USA), which reacts with the unbound cysteine on the peptide to form a fluorescent complex. The extent of peptide depletion was determined by measuring the fluorescence signal from the mBrB-peptide complex. Depletion exceeding the threshold of 13.89% was considered positive (*i.e.*, having skin-sensitizing potential). Second-order rate constants (k in min^{-1} mM^{-1}) were calculated for each of the six time points based on the slope of the depletion vs. concentration. This rate constant was transformed to k in M^{-1} s^{-1}, and the logarithm was used. The highest value observed across all time points was determined to be the log k_{max} for the test chemical. A test chemical with a log $k_{max} \geq -2.0$ was classified as GHS subcategory 1A, while a log $k_{max} < -2.0$ classified a chemical as GHS subcategory 1B/not classified.

At least one independent experiment that meets the data acceptance criteria is required to establish a final classification. The assay results were accepted as valid if: 1) the log k_{max} of the positive control at 90-min exposure was within the range of −1.75 to −1.40 $M^{-1}s^{-1}$. If no log k_{max} was obtained at 90 min, then the log k_{max} at 150-min exposure was taken into account and was within the range of −1.90 to −1.45$M^{-1}s^{-1}$; and 2) the variance of the 12 negative control values of a plate was <12.5% for 5 of the 6 time points.

Data from at least one valid experiment were obtained for NNDMA, ACMO, IBOA, and SO60.

2.3. The KeratinoSens™ assay

The KeratinoSens™ Assay (KS) is a reporter-cell assay that evaluates the activation of the Keap1-Nrf2-antioxidant/electrophile response element (ARE)-dependent pathway in luciferase gene-transfected HaCaT keratinocytes (*i.e.*, KeratinoSens™ cells) following a 48-h test chemical exposure (Emter et al., 2010; Natsch and Emter, 2008). The Keap1-Nrf2-ARE regulatory pathway is involved in developing skin sensitization (El Ali et al., 2013; Kim et al., 2008; van der Veen et al., 2013).

The KS was conducted according to the OECD Test Guideline 442D (OECD, 2022b) and the EURL ECVAM DB-ALM Protocol n° 155: KeratinoSens™. Briefly, KeratinoSens™ cells were seeded at 10,000 cells/-well in three 96-well plates for assessing luciferase induction and one 96-well plate for evaluating cytotoxicity. The cells were pre-cultured for 24 h before test chemical exposure. To create a 100x plate, 200 mM stock solutions were prepared for NNDMA, ACMO, and IBOA in dimethyl sulfoxide (DMSO) and, due to limited solubility, a 1 mM stock in DMSO was prepared for SO60. Eleven serial 1:2 dilutions were made from the NNDMA, ACMO, and IBOA stock solutions in DMSO, resulting in 12 concentrations ranging from 200 mM to 0.098 mM. Eleven serial 1:1.3 dilutions were made from the SO60 stock dilution in DMSO, resulting in 12 concentrations ranging from 1 mM to 0.06 mM. The dilutions in the 100x plate were diluted 25-fold in culture medium to create a 4x plate with 12 concentrations ranging from 8000 μM to 3.9 μM for NNDMA, ACMO, and IBOA, and 40 μM–2.23 μM for SO60. The pre-cultured cells were dosed with test chemicals from the 4x plate at a 4-fold dilution, resulting in final in-well concentrations of 2000, 1000, 500, 250, 125, 62.5, 31.3, 15.6, 7.82, 3.91, 1.95, and 0.98 μM for NNDMA, ACMO and IBOA. The final in-well concentrations for SO60 were 10, 7.69, 5.92, 4.55, 3.50, 2.69, 2.07, 1.59, 1.23, and 0.94 μM. Following a 48-h test chemical exposure, cell viability (1 plate) was assessed by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) method, and luciferase induction (3 plates) was measured in cell lysates by luminescence detection of the conversion of adenosine triphosphate (ATP) and a luciferin substrate into light.

The test chemical concentrations, which resulted in a 1.5-fold (EC1.5) and 3-fold (EC3.0) increase in luciferase induction relative to the solvent control, were calculated by linear interpolation from the concentration-response curve. In addition, the test chemical concentrations, which produced a 30% and 50% decrease in cell viability (IC_{30} and IC_{50}, respectively), were also calculated from the MTT concentration-response data. A test chemical was considered positive (*i.e.*, having skin sensitizing potential) if the luciferase induction is ≥ 1.5-fold compared to the solvent control luciferase activity and the EC1.5 value is below 1000 μM in at least 2 independent experiments. In addition, the lowest concentration with an induction ≥1.5 fold (*i.e.*, the EC 1.5) must have greater than 70% cellular viability. Additional considerations to the prediction model were used when chemicals that are not soluble at an in-well concentration of 1000 μM: a chemical can be classified as positive if the luciferase induction threshold of 1.5-fold over solvent control is obtained at a lower, non-cytotoxic concentration where the chemical is soluble.

At least two independent experiments that meet the data acceptance criteria and are concordant are required to establish a final classification. A definitive assay was considered valid when the luciferase gene

Table 2
kDPRA and KS data.

Chemical	kDPRA Log k_{max} ($M^{-1}s^{-1}$)	KS Trial	EC1.5 (μM)	EC1.5 Geo Mean (μM)	EC3.0 (μM)	EC3.0 Geo Mean (μM)	IC_{30} (μM)	IC_{30} Geo Mean (μM)	IC_{50} (μM)	IC_{50} Geo Mean (μM)	I_{max}
NNDMA	−3.15	1	531	485.56	NA[a]	NA[a]	>2000	>2000	>2000	>2000[b]	2.83
		2	444		NA[a]		>2000		>2000		2.94
ACMO	−2.11	1	123	97.76	470.15	496.17	1264	1247	1626	1592.66	60.1
		2	77.7		523.62		1230		1560		65.4
IBOA	−1.28	1	5.21	4.38	15.72	16.0	35.1	34.9	42.9	42.80	18.6
		2	3.69		16.28		34.7		42.7		16.7
SO60[c]	−2.27	1	1.29	1.47	3.93	3.91	3.31	3.16	4.05	4.17	3.45
		2	1.85		3.50		3.66		4.82		3.72
		3	1.34		4.33		2.61		3.72		3.15

[a] NA = Not applicable. Luciferase activity was not induced above the EC3.0 threshold. The data input used for the regression model was the default value of 4000 μM.
[b] The test chemical was not cytotoxic at the highest concentration tested (*i.e.*, 2000 μM). The data input used for the regression model was the default value of 4000 μM.
[c] Due to limited solubility, the maximum stock concentration for the kDPRA was 5 mM, and the highest in-well concentration tested in the KS was 10 μM.

induction by the positive control was ≥1.5 in comparison to the solvent control in at least one concentration, and the EC1.5 fell within two standard deviations of the historical mean. Further, a definitive assay was considered valid when 1) the coefficient of variation of the solvent controls used in the luminescence readings was <20%; 2) the positive control was positive and resulted in an EC1.5 value < 64 μM; and 3) the positive control produced a > 2-fold luciferase induction at 64 μM.

Data from two valid experiments were obtained for NNDMA, ACMO, and IBOA. Data for three valid experiments were available for SO60.

2.4. Determination of pEC3 values

Several regression models were developed to calculate a predicted Local Lymph Node Assay (LLNA) EC3 value (pEC3), which can be used as PoD in the risk assessment (Natsch, 2023; Natsch and Gerberick, 2022a, 2022b). Regression analysis can be conducted using partial experimental data (kDPRA and KS or h-CLAT; KS and h-CLAT) or comprehensive data (all three tests). This investigation used data from the kDPRA and KS, along with the MW and VP. Except for the MW, all equations use log-transformed, normalized data inputs as described below.

2.4.1. Data inputs, log transformation, and normalization

The MW was used without further modification. The average experimentally or predicted derived VP in Pa was used. The data were log-transformed and normalized as follows:

$$Log\ VP_{norm} = Log(VP) - 1$$

The key data derived from the kDPRA used in the regression model is the logarithm of the maximal reaction rate in $s^{-1}M^{-1}$ (log k_{max}). The data were normalized using the following equation:

$$Logk_{\max\ norm} = Logk_{max} + 3.5$$

Three data inputs from the KS were used in the regression models. The IC_{50} value, the EC1.5 and/or EC3.0 values. For chemicals with no cytotoxicity at the maximum tested concentration of 2000 μM, the numerical IC_{50} was set to an arbitrary value of 4000 μM. Similarly, if the luciferase gene was not induced above a given threshold, the EC1.5 or EC3.0 values were set to 4000 μM. The geometric mean of the independent assays was used. The three parameters were linearized by log transformation and normalized by multiplication by −1 and the addition of the constant Log(4000) as shown below:

$$LogIC50_{norm} = -1 \times Log(IC50_{KS}) + Log(4000)$$

$$LogEC1.5_{norm} = -1 \times Log(EC1.5_{KS}) + Log(4000)$$

$$LogEC3.0_{norm} = -1 \times Log(EC3.0_{KS}) + Log(4000)$$

2.4.2. The regression models

While several regression models were available to calculate a pEC3, selecting model(s) to use was based on the available data and other considerations (Natsch and Gerberick, 2022a). This investigation used the global regression model integrating kDPRA data with KS data since it is the key model for chemicals with available kDPRA and KS data only. The pEC3 was calculated using the following equation:

$$pEC3 = 0.42 + 0.40 \times Log\ k_{max\ norm} + 0.15 \times Log\ EC1.5_{norm} + 0.36 \times Log\ IC50_{norm} - 0.21 \times Log\ VP_{norm}$$

The calculated pEC3 value was converted to an EC3 value in % using the equation below.

$$EC3 = MW/10^{\wedge}pEC3$$

The % EC3 was converted to a concentration per unit area (*i.e.*, μg/cm^2) using a conversion factor of 250 (*e.g.*, an EC3 of 1% is equivalent to 250 μg/cm^2) (Basketter et al., 2005).

3. Results

3.1. N, N-dimethylacrylamide (NNDMA)

Existing *in vivo* data, a negative guinea pig maximization test at a topical induction concentration of 25% was not sufficient to derive a PoD for NNDMA (ECHA dossier: https://echa.europa.eu/lv/registration-dossier/-/registered-dossier/13166/7/5/2/?documentUUID=509849a8-1630-4ea3-8cb5-6538bc209846). NNDMA was tested in the kDPRA with the standard stock solution concentration of 20 mM, resulting in final tested concentrations of 0.31, 0.63, 1.25, 2.5, and 5 mM. The test run met all acceptance criteria, including those for the cinnamic aldehyde positive control (data not shown). The threshold for positivity, 13.89% peptide depletion, was exceeded only at the 1440-min time point (data not shown). The resulting log k_{max} was calculated to be −3.15 (Table 2). According to the kDPRA prediction model, NNDMA would be classified as GHS subcategory 1B/not classified. However, since peptide depletion >13.89% was observed, NNDMA would be considered to have peptide reactivity.

NNDMA was tested in two independent KS trials, which were concordant and met all acceptance criteria (data not shown). NNDMA was not cytotoxic, with viability >90% at the highest in-well concentration of 2000 μM in both trials (data not shown). Therefore, neither an IC_{30} nor IC_{50} could be calculated. The concentration required to induce a 1.5-fold increase in luciferase activity compared to the solvent control (*i.e.*, EC1.5) calculated from the concentration-response curve was 531 and 444 μM for trials 1 and 2, respectively, with a geometric mean of 485.56 μM. Induction at 3-fold of the solvent control (*i.e.*, EC3.0) was not obtained in either trial. Therefore, an EC3.0 could not be calculated.

Table 3
Regression model data inputs and pEC3 values.

Chemical	GHS Category	MW (g/mol)	Log VP_{norm}	kDPRA Log $k_{max\ norm}$	KS Log $EC1.5_{norm}$	KS Log $EC3.0_{norm}$	KS Log $IC50_{norm}$	pEC3	EC3 (%)	EC3 (μg/cm^2)
NNDMA	1B	99.13	1.038	0.35	0.9158	0	0	0.47	33.30	8325
ACMO	1	141.17	0.0065	1.93	1.612	0.9064	0.3999	1.36	6.14	1535
IBOA	1A	208.30	0	2.22	2.961	2.398	1.971	2.46	0.72	180
SO60	1B	270.28	0	1.23	3.435	3.010	2.982	2.50	0.86	215

The results of the KS would predict NNDMA as activating keratinocytes.

For the determination of a pEC3, the data input values used were the MW, VP (in Pa), log k_{max} from the kDPRA, the KS EC1.5, and the default value of 4000 μM for both the KS IC_{50} and EC3.0 since NNDMA was not cytotoxic and an EC3.0 could not be calculated. The MW, the normalized log k_{max} from the kDPRA, the log-transformed normalized values for the VP and the KS IC_{50}, and EC1.5 were used in the global regression model, resulting in a pEC3 of 0.47 (Table 3). The pEC3 was converted to an EC3 of 33.3% or 8325 μg/cm^2 (Table 3). It should be noted that the VP will not play a role since the EC3 will be reduced to 20% so NNDMA would still be a weak sensitizer.

3.2. 4-Acryloylmorpholine (ACMO)

The existing *in vivo* data for ACMO were inconclusive, with one positive guinea pig maximization test at a topical induction concentration of 30%, one negative Buehler guinea pig test using neat test material for induction, and an LLNA that was negative up to 25% (ECHA dossier: https://echa.europa.eu/lv/registration-dossier/-/registered-dossier/16358/7/5/1). The existing *in vivo* data could not be used to derive a PoD. ACMO was tested in the kDPRA with the standard stock solution concentration of 20 mM, resulting in final tested concentrations of 0.31, 0.63, 1.25, 2.5, and 5 mM. The test run met all acceptance criteria (data not shown). The threshold for positivity, 13.89% peptide depletion, was exceeded at the 150-, 210-, and 1440-min time points (data not shown). The resulting log k_{max} was calculated to be −2.11 (Table 2). According to the kDPRA prediction model, ACMO would be classified as GHS subcategory 1B/not classified. However, since peptide depletion >13.89% was observed at three time points, ACMO would be considered to have peptide reactivity.

ACMO was tested in two independent KS trials, which were concordant and met all acceptance criteria (data not shown). AMCO was cytotoxic at the highest concentration tested, 2000 μM, in both trials (data not shown), which resulted in calculated IC_{30} and IC_{50} geometric means of 1247 μM and 1593 μM, respectively (Table 2). The concentration required to induce a 1.5-fold increase in luciferase activity compared to the solvent control (*i.e.*, EC1.5) calculated from the concentration-response curve was 123 and 77.7 μM for trials 1 and 2, respectively, resulting in a geometric mean EC1.5 of 97.76 μM. Induction at 3 times the solvent control, the EC3.0 was 470.15 μM for trial 1 and 523.62 μM for trial 2, with a geometric mean of 496.17 μM (Table 2). The results of the KS would classify AMCO as activating keratinocytes.

To determine a pEC3, the data input values used were the MW, VP (in Pa), log k_{max} from the kDPRA, the KS IC_{50}, EC1.5, and EC3.0. The MW, the normalized log k_{max} from the kDPRA, and the log-transformed normalized values for the VP, the KS IC_{50}, and EC1.5 were used in the global regression model, resulting in a pEC3 of 1.36 (Table 3). The pEC3 was converted to an EC3 of 6.14% or 1535 μg/cm^2 (Table 3).

3.3. Isobornyl acrylate (IBOA)

An existing LLNA for IBOA was positive at all concentrations, with 5% as the lowest tested (ECHA dossier: https://echa.europa.eu/lv/registration-dossier/-/registered-dossier/14494/7/5/1). The existing *in vivo* data could not be used to derive a PoD. IBOA was tested in the kDPRA with the standard stock solution concentration of 20 mM, resulting in final tested concentrations of 0.31, 0.63, 1.25, 2.5, and 5 mM. The test run met all acceptance criteria (data not shown). The threshold for positivity, 13.89% peptide depletion, was exceeded at all time points except for the 10-min time point (data not shown). The resulting log k_{max} was calculated to be −1.28 (Table 2). According to the kDPRA prediction model, IBOA would be classified as a GHS subcategory 1A and have skin sensitization potential (sensitizer).

IBOA was tested in two independent KS trials, which were concordant and met all acceptance criteria (data not shown). IBOA was cytotoxic in both trials (data not shown), resulting in calculated IC_{30} and IC_{50} geometric means of 34.9 μM and 42.8 μM, respectively (Table 2). The concentration required to induce a 1.5-fold increase in luciferase activity compared to the solvent control (*i.e.*, EC1.5) calculated from the concentration-response curve was 5.21 μM for trial 1 and 3.69 μM for trial 2, resulting in a geometric mean EC1.5 of 4.38 μM. Induction at 3 times the solvent control, the EC3.0 was 15.72 μM for trial 1 and 16.28 μM for trial 2, with a geometric mean of 16.0 μM (Table 2). The results of the KS would classify IBOA as activating keratinocytes.

To determine a pEC3, the data input values used were the MW, VP (in Pa), log k_{max} from the kDPRA, the KS IC_{50}, EC1.5, and EC3.0. The MW, the normalized log k_{max} from the kDPRA, and the log-transformed normalized values for the VP, the KS IC_{50}, and EC1.5 were used in the global regression model, resulting in a pEC3 of 2.46 (Table 3). The pEC3 was converted to an EC3 of 0.72% or 180 μg/cm^2 (Table 3).

3.4. Solvent Orange 60 (SO60)

While positive in human diagnostic patch tests, SO60 was negative in a LLNA up to 10% (ECHA dossier: https://echa.europa.eu/lv/registration-dossier/-/registered-dossier/11457/7/5/1). Therefore, no PoD could be derived from the existing data. SO60 had limited phosphate buffer and acetonitrile solubility at the standard 20 mM stock concentration used in the kDPRA. However, it was slightly more soluble in acetonitrile, which was selected as the solvent of choice, and a 5 mM stock solution was used, producing final reaction mixture concentrations of 1.25, 0.63, 0.31, 0.16, and 0.08 mM. The kDPRA test run met all acceptance criteria, including those for the cinnamic aldehyde positive control (data not shown). The threshold for positivity, 13.89% peptide depletion, was exceeded at the 10-, 90-, and 1440-min time points (data not shown). The resulting log k_{max} was calculated to be −2.27 (Table 2). This log k_{max} would classify SO60 as 'Not categorized as subcategory 1A' according to the kDPRA prediction model. However, since a 20 mM stock concentration could not be used for testing, the binary classification according to the kDPRA prediction model was considered inconclusive. Since peptide depletion >13.89% was observed, SO60 would be considered to have peptide reactivity.

For the KS assay, SO60 had limited solubility in the DMSO solvent and could not be tested at the standard 200 mM stock solution. Thus, a 1 mM stock solution was evaluated. SO60 was tested in three independent KS trials, which were concordant and met all acceptance criteria (data not shown). SO60 was cytotoxic in all trials (data not shown), resulting in calculated IC_{30} and IC_{50} geometric means of 3.13 μM and 4.17 μM, respectively (Table 2). The concentration required to induce a 1.5-fold increase in luciferase activity compared to the solvent control (*i.e.*, EC1.5) calculated from the concentration-response curve was 1.29 μM

for trial 1, 1.85 μM for trial 2, and 1.34 μM for trial 3, resulting in a geometric mean EC1.5 of 1.47 μM. Induction at 3 times the solvent control, the EC3.0 was 3.93 μM for trial 1, 3.50 μM for trial 2, and 4.33 μM for trial 3, with a geometric mean of 3.91 μM (Table 2). Although the SO60 was not completely soluble, it produced luciferase induction >1.5-fold relative to the solvent controls and at concentrations with viability >70%, and therefore, was predicted to activate keratinocytes according to the test method prediction model in consideration of the solubility limitation.

To determine a pEC3, the data input values used were the MW, VP (in Pa), log k_{max} from the kDPRA, the KS IC_{50}, EC1.5, and EC3.0. The MW, the normalized log k_{max} from the kDPRA, and the log-transformed normalized values for the VP and the KS IC_{50} and EC1.5 were used in the global regression model, resulting in a pEC3 of 2.50 (Table 3). The pEC3 was converted to an EC3 of 0.86% or 215 μg/cm^2 (Table 3).

4. Discussion

In recent years, there have been case reports of materials used in the manufacturing of consumer and medical wearable products causing ACD, including Solvent Orange 60 (SO60), Isobornyl acrylate (IBOA), N, N- Dimethylacrylamide (NNDMA) and Acryloylmorpholine (ACMO) (Ahrensbøll-Friis et al., 2022; Gatica-Ortega et al., 2021; Herreros-Montejano et al., 2022; Joyanes Romo et al., 2022; Mowitz et al., 2019; Pesonen and Suuronen, 2020). This study evaluated these four compounds using two adopted OECD test methods, the kDPRA (Natsch et al., 2020; Wareing et al., 2020) and the KS assay (Emter et al., 2010), to determine PoD values for potential use in a risk assessment approach. Other regression models are available that use data from all three TGs, kDPRA, KS, and h-CLAT, or two TGs, KS and h-CLAT, or kDPRA and h-CLAT (Natsch and Gerberick, 2022a). Although this paper is focused on a POD for risk assessment purposes the data can be used in the 2 out of 3 approach (Natsch and Gerberick, 2022b).

SO60 is a perinone-type dye found in plastic frames and temple tips of eyeglasses, goggles, gloves, and helmets. The first case of contact allergy was reported in 1999 (Shono and Kaniwa, 1999) and later in 2011 (Yeo et al., 2011), leading to manufacturers' reduced use of the ingredient in Japan. However, strong allergic reactions have recently been reported to be caused by SO60 being used in eyeglasses (Kawakami et al., 2021; Shono et al., 2019; Uter et al., 2020). In addition to being associated with eyeglasses, SO60 has also been shown to cause ACD in patients using protective gloves (Pesonen and Suuronen, 2020). SO60 has been found in brown, red, pale pink, and tortoiseshell plastic spectacle frames and earpieces. It is believed that unbound SO60 leaches out of the plastic articles and transudes into the skin (Shono and Kaniwa, 1999; Yeo et al., 2011). The EC3 value predicted for SO60 was 0.86% or 215 μg/cm^2 (Table 3). Thus, the skin sensitization potency of SO60 would be categorized as a strong sensitizer based on previously published sensitization categories (Na et al., 2022).

NNDMA and IBOA are well known to be present in adhesives used in wearable medical devices such as glucose sensors or insulin pumps. Numerous reports demonstrate strong contact allergic responses in patch test subjects to IBOA and NNDMA (Herman et al., 2018; Mowitz et al., 2019; Svedman et al., 2021; Ulriksdotter et al., 2020; Uter et al., 2020). ACD caused by glucose sensors has become an increasing problem. These wearable devices are worn for a prolonged time, which can increase the likelihood of an individual acquiring ACD. Contact allergies to IBOA, colophony, ethyl cyanoacrylate, and NNDMA have been reported (Hyry et al., 2019). It is believed that IBOA is the most relevant culprit in causing allergy in individuals wearing glucose sensors (Herman et al., 2017; Hyry et al., 2019; Joyanes Romo et al., 2022). Thirty-nine of 1036 patients (3.8%) had ACD due to IBOA, and only two patients benefited from using barrier films (Pyl et al., 2020).

The EC3 value predicted for IBOA was 0.72% or 180 μg/cm^2 (Table 3). This would categorize the skin sensitization potency of IBOA as a strong sensitizer based on previously published sensitization categories (Na et al., 2022). Interestingly, in analyses of adhesive patches from glucose sensors, the surface concentrations of IBOA were between 0.2 and 6 μg/cm^2. As a comparison, the surface concentration of IBOA in a 0.1% petrolatum preparation tested in an 8 mm Finn Chambers AQUA is 40 μg/cm^2 (Svedman et al., 2021). Other analyses of patch-based medical devices containing IBOA, performed according to ISO 10993, found that these products can leach IBOA at concentrations (15 mg/L) that result in a sensitizer classification when tested in the Keratinosens™ (Fink et al., 2021).

The EC3 value predicted for NNDMA was 33.3% or 8325 μg/cm^2 (Table 3). This would categorize the skin sensitization potency of NNDMA as a weak sensitizer based on previously published sensitization categories (Na et al., 2022). The lower sensitization potency could explain IBOA being the most observed culprit associated with glucose sensors causing ACD when other adhesive compounds are present.

Acryloylmorpholine (ACMO) is a photoinitiator and polyurethane oligomer used for wearable devices and smartphone glass protectors. Industrial uses of this molecule include adhesives and sealants, coating products, inks, toners, pharmaceuticals, photo-chemicals, nail products, manufacturing of plastic products, ultraviolet curable resins (as a reactive diluent because of its low viscosity and high curability), and oil field polymers (Herreros-Montejano et al., 2022). Occupational and non-occupational ACD to ACMO have been reported related to the manufacturing and use of smartphone devices (Gatica-Ortega et al., 2021; Herreros-Montejano et al., 2022; Otero-Alonso et al., 2020). The subjects showed strong positive patch test responses to 0.5% ACMO but not to IBOA (Gatica-Ortega et al., 2021). The authors state whether there are cross-reactions with other adhesive materials like NNDMA is unclear (Herreros-Montejano et al., 2022). The EC3 predicted for ACMO was 6.14% or 1535 μg/cm^2 (Table 3). This would categorize the skin sensitization potency of ACMO as a moderate sensitizer based on previously published sensitization categories (Na et al., 2022).

It is critical to have tools completely dependent on using animal-free methods to generate data for risk assessors responsible for assessing the skin sensitization risk of new chemical entities or chemicals lacking sufficient data. The regression models used in this investigation use input data from OECD-validated NAMs, including the recently accepted kDPRA (Na et al., 2022; OECD, 2022a) and the KS (OECD, 2022b, c). The PoD values obtained from these regression models can be used to assess skin sensitization risk (Api et al., 2020; Bernauer et al., 2021; Gilmour et al., 2020). Importantly, it is critical to understand the sources of uncertainty in the NAM datasets used to calculate a PoD. For these pEC3 linear regression models, a factor of 2–3 is proposed to refine the PoD, which accounts for the models' uncertainty and considers additional available data, e.g., suitable analogs for use in read-across. Thus, the PoD values obtained for SO60, IBOA, NNDMA, and ACMO would be divided by a factor of 3 before conducting an exposure-based risk assessment (Natsch et al., 2018; Natsch and Gerberick, 2022a).

The four compounds evaluated in this study have been associated with causing ACD in subjects using wearable products (Ahrensbøll-Friis et al., 2022; Gatica-Ortega et al., 2021; Herreros-Montejano et al., 2022; Joyanes Romo et al., 2022; Mowitz et al., 2019; Pesonen and Suuronen, 2020). For each of them, insufficient data was available to determine their skin sensitization potency for risk assessment. Although important clinical patch test data was available, it is impossible to gauge the skin sensitization potency from either the severity of the reactions or the prevalence of responses observed in the patch test clinics. Therefore, two OECD-adopted NAMs for skin sensitization that also provide continuous quantitative data were conducted on each ingredient to establish a PoD, which, after adjustment to account for uncertainty in the data, can be used in a quantitative skin sensitization risk assessment.

Establishing the skin sensitization potency and determining a PoD is required for conducting a thorough risk assessment to ensure consumer and worker safety. The PoD may be defined using existing data, including *in vivo* studies (*i.e.*, Local Lymph Node Assay) and/or human test data (*i.e.*, Human Maximization Test and Human Repeat Insult Patch

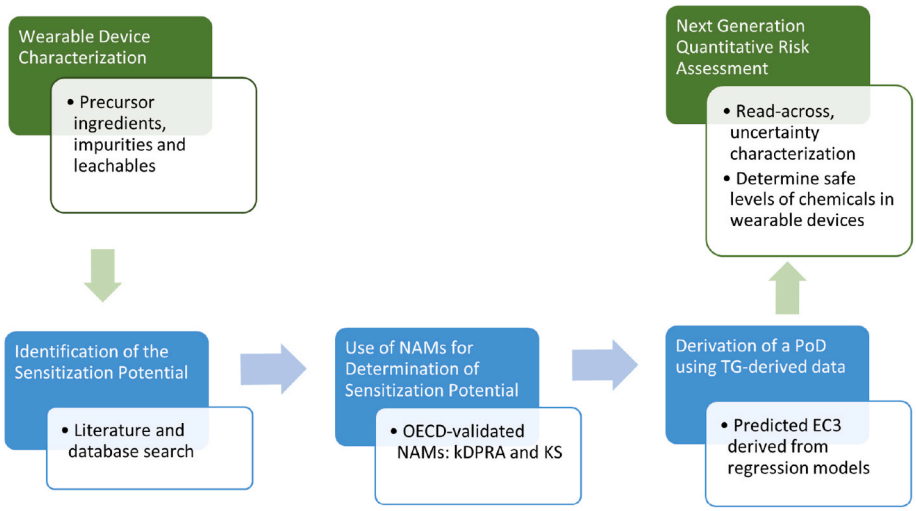


Fig. 1. Proposed framework to derive NAMs-derived POD of wearable devices.

Test) (Api et al., 2008; Na et al., 2022). The need to have NAMs and Defined Approaches (DAs) available to help establish the skin sensitization potency of chemicals is critical for assessing chemicals with insufficient or no data. Specifically, determining a PoD is required for conducting risk assessments on new chemical entities for which only non-animal information is available (Api et al., 2020; Bernauer et al., 2021; Gilmour et al., 2020).

Based on the procedures used in this study, a proposed framework to establish NAMs-derived PoD for skin sensitization risk assessment of wearable devices is shown in Fig. 1. Wearable devices, like medical devices, may also lead to skin contact allergy due to their chemical components and those used in their manufacture (e.g., catalysts, initiators, cross-linking agents, processing aids) and any impurities and leachables. Chemical characterization is needed to identify these chemicals and to evaluate their skin sensitization potential. Without sufficient data, the chemicals' skin sensitization potential can be determined via OECD-adopted NAMs, which provide continuous potency data (e.g., kDPRA, KS, and h-CLAT). A PoD in the form of a predicted EC3 value is then derived for chemicals identified as sensitizers using the linear regression models method. Along with exposure data and appropriate uncertainty factors, safe levels of sensitizing chemicals to be used in wearable devices can be determined based on the predicted EC3. This strategy demonstrates that chemical risk assessment can be conducted without the use of animal models.

5. Conclusions

Regression models have been developed to derive a PoD for skin sensitization risk assessment using only NAMs data. These models are a valuable tool for conducting next generation risk assessments on new or existing chemicals that have no or only limited information available and testing is needed. In this study, PoD values were determined for four materials (Solvent Orange, Isobornyl acrylate, N, N- Dimethylacrylamide, and Acryloylmorpholine) that are used in wearable devices and known to be associated with causing skin allergy. The global regression model integrating kDPRA data with KS data was used to derive the pEC3 values in this study since it is the key model for chemicals with kDPRA and KS data only. This regression model demonstrates that it is possible to derive a PoD for use in risk assessment without the generation of *in vivo* data. Thus, it serves as a true replacement for animal testing.

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Nakul Ruparel: Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Conceptualization. **Argel Islas-Robles:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Investigation, Formal analysis, Conceptualization. **Allison Hilberer:** Writing – review & editing, Supervision, Project administration, Formal analysis. **Kayla Cantrell:** Writing – review & editing, Investigation, Formal analysis. **Megan Madrid:** Investigation, Formal analysis. **Cindy Ryan:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Conceptualization. **G. Frank Gerberick:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Conceptualization. **Ravi Persaud:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The Institute for In Vitro Sciences Inc. (IIVS) is a contract research organization. AIR, AH, KC, and MM are paid employees of IIVS. CR is a paid independent consultant to IIVS. GFG is a paid independent consultant to IIVS and Apple Inc.

Data availability

Data will be made available on request.

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