Evaluation of Substances of Regulatory Interest Using Non-Animal Skin Sensitization Test Methods

National Institutes of Health
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National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

National Institute of Environmental Health Sciences
National Institutes of Health
Department of Health and Human Services

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FOREWORD

The National Toxicology Program (NTP), established in 1978, is an interagency program within the Public Health Service of the U.S. Department of Health and Human Services. Its activities are executed through a partnership of the National Institute for Occupational Safety and Health (part of the Centers for Disease Control and Prevention), the U.S. Food and Drug Administration (primarily the National Center for Toxicological Research), and the National Institute of Environmental Health Sciences (part of the National Institutes of Health), where the program is administratively located. NTP offers a unique venue for the testing, research, and analysis of agents of concern to identify toxic and biological effects, provide information that strengthens the science base, and inform decisions by health regulatory and research agencies to safeguard public health. The Division of Translational Toxicology within NTP works to develop and apply new and improved methods and approaches that advance toxicology and better assess health effects from environmental exposures.

The NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) is an NTP office focused on the development and evaluation of alternatives to animal use for chemical safety testing. NICEATM was established by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) Authorization Act of 2000 (42 U.S.C. 285 *l*-3) to provide support to ICCVAM. NICEATM and ICCVAM work collaboratively to evaluate new and improved testing approaches applicable to the needs of U.S. federal agencies.

NICEATM publishes reports of its test method development and evaluation activities in the scientific literature. Through NTP, NICEATM also issues reports of ICCVAM test method evaluations and other communications and makes these available on the NTP website, where they are available free of charge. Data for these studies are included in NTP's Chemical Effects in Biological Systems database.

For questions about the reports and studies, please contact NICEATM.

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Integrated approaches for assessing skin sensitization potential and predicting potency category leverage the combination of multiple types of methods to overcome predictive limitations of the individual tests. Integrated approaches encompass a variety of strategies to consider multiple types of information about a substance to arrive at a hazard characterization decision. One of these, known as the defined approach (DA), uses predetermined data sources to arrive at an outcome without the use of expert judgment. This is accomplished through the application of the results of the methods used in the DA in an explicitly defined data interpretation protocol. Skin sensitization DAs combine non-animal tests that align with multiple key events in an adverse outcome pathway for skin sensitization to inform on chemical hazard and potency. This report evaluates the performance of individual in chemico, in vitro, and in silico methods for predicting skin sensitization hazard or potency by comparing predictions derived from testing within the individual methods to those of the murine local lymph node assay (LLNA) and to those of three DAs: the Key event (KE) 3/1 Sequential Testing Strategy (STS) from Nukada et al. (2013), the 2 out of 3 (2o3) strategy from Bauch et al., 2012; and Urbisch et al., 2015, and the Integrated Testing Strategy (ITSv2) from Takenouchi et al. (2015).

Recognizing a need to better characterize or expand upon the types of substances that can be assessed with these methods, the National Toxicology Program Interagency Center for the Evaluation of Alternative Methods (NICEATM) solicited nominations of substances relevant to regulatory requirements for skin sensitization from member agencies of the Interagency Coordinating Committee on the Validation of Alternative Methods. NICEATM then coordinated testing of nominated substances using three non-animal skin sensitization tests: the direct peptide reactivity assay (DPRA), KeratinoSens™ test, and human cell line activation test (h-CLAT). Each of these assays is internationally accepted through test guidelines issued by the Organisation for Economic Co-operation and Development (OECD) test guidelines. The prediction of skin sensitization hazard for each substance was also determined by using the in silico read-across algorithms provided by the OECD QSAR Toolbox. Animal (LLNA) and human (predictive patch test) reference data were obtained from published literature, publicly available databases, or directly from nominating agencies for comparison with the non-animal results.

In all, 181 substances were assessed in the three in vitro methods, with results both evaluated individually and used as input into the DAs. Predictions derived from both the individual methods and from the DAs were evaluated for concordance with one another and also with human and LLNA reference data. Concordance between the methods for all tested substances varied from ~ 56% to 83%, while similar concordance assessments of the individual methods against the LLNA reference data varied from 30% to 93%. Concordance of the DAs with each other varied from 70% to 92%. DAs were only 24% to 51% concordant with LLNA reference data. Human reference data were available for 25 substances. Human reference data were 52% to 78% concordant with individual methods and 42% to 47% concordant with the DAs. Similar concordance values were found between the human reference data and the LLNA. Overall, the concordance of the hazard and potency categorization predictions was higher among the DAs

than between either human or LLNA reference data. Concordance of potency predictions between the DAs and human reference data was generally higher than concordance between the DAs and the LLNA reference data. This suggests that the DAs are overall better predictors of human sensitization hazard and potency than the LLNA.

LIST OF ACRONYMS AND ABBREVIATIONS

2o3	2 out of 3
AOP	Adverse outcome pathway
ARE	Nrf2-dependent antioxidant response element
BRT	Burleson Research Technologies, Inc
BRTIV	BRT In Vitro ID for test substances
CASRN	Chemical Abstracts Service Registry Number
CCTE	U.S. Environmental Protection Agency Center for Computational Toxicology and Exposure
CPSC	U.S. Consumer Product Safety Commission
CV75	Concentration needed to produce viability of 75%
DAs	Defined approaches
DASS	Defined approaches for skin sensitization
DPRA	Direct peptide reactivity assay
DTT	Division of Translational Toxicology, National Institute of Environmental Health Sciences
EC150	Effective concentration of a test substance that produces a 150% increase in the expression of the CD86 cell surface marker
EC200	Effective concentration of a test substance that produces a 200% increase in expression of the CD54 cell surface marker
EPA	U.S. Environmental Protection Agency
FDA	U.S. Food and Drug Administration
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GPMT	Guinea pig maximization test
h-CLAT	Human cell line activation test
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
ICE	Integrated Chemical Environment
ITS	Integrated testing strategy
KE	Key event
KE 3/1 STS	Key events 3 and 1 sequential testing strategy
LLNA	Murine local lymph node assay

MIT	Minimum induction threshold
NAMs	New approach methodologies
NA	Not applicable
NC	Not classified (GHS hazard classification)
NICEATM	National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods
NTP	National Toxicology Program
OECD	Organisation of Economic Co-operation and Development
OPERA	Open Structure-activity/property Relationship App
OPP	U.S. Environmental Protection Agency Office of Pesticide Products
OPPT	U.S. Environmental Protection Agency Office of Pollution Prevention and Toxics
QSAR	Quantitative structure-activity relationships
SMILES	Simplified Molecular Input Line Entry System

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1. INTRODUCTION

Recent advances in the development of alternative or new approach methodologies (NAMs) for skin sensitization testing have made it possible to consider the use of several test methods as replacements for traditional in vivo methods such as the guinea pig maximization test, the Buehler test, or the murine local lymph node assay (LLNA). In vitro/in chemico methods such as the direct peptide reactivity assay (DPRA), the KeratinoSens™ assay, and the human cell line activation test (h-CLAT) can be used as inputs to defined approaches (DAs) to evaluate substances for skin sensitization hazard and potency classification. DAs use predetermined data sources to arrive at an outcome without the use of expert judgment. This is accomplished through the application of the results of the methods used in the DA in an explicitly defined data interpretation protocol.

Guideline on Defined Approaches for Skin Sensitisation (DASS), Guideline No. 497, issued in 2021 by the Organisation for Economic Co-operation and Development (OECD), was the first internationally harmonized guideline to describe a non-animal approach that can be used to fully replace an animal test to identify skin sensitizers. OECD Guideline 497 describes two validated DAs to classify substances for skin sensitization hazard and/or potency: the 2 out of 3 (2o3) and the Integrated Testing Strategy (ITS). A third DA, Key Event 3/1 Sequential Testing Strategy (KE 3/1 STS), has been accepted by the U.S. Environmental Protection Agency (EPA) for skin sensitization hazard classification (US EPA, 2018).

1.1. Background

The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM) is an office within the National Institute of Environmental Health Sciences Division of Translational Toxicology (DTT). NICEATM is focused on the development and evaluation of alternatives to animal use for chemical safety testing. In response to a recognized need to expand the number and types of substances with available in vitro skin sensitization testing data, NICEATM embarked on a project to evaluate NAMs for skin sensitization potential. They identified three in chemico or in vitro test methods to be used by the DTT contract testing laboratory, Burleson Research Technologies, Inc. (BRT) to test a variety of substances. The test methods used for the evaluation were the first NAMs for skin sensitization evaluation that were described in test guidelines from OECD: DPRA (in chemico), KeratinoSens (in vitro), and h-CLAT (in vitro).

NICEATM requested nominations for substances to test from the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) member agencies. ICCVAM is composed of 18 U.S. federal regulatory and research agencies that require, use, generate, or disseminate toxicological and safety testing information. DTT, the U.S. Consumer Product Safety Commission (CPSC), the U.S. Food and Drug Administration (FDA), and three offices of EPA — Office of Pesticide Products (OPP), Office of Pollution Prevention and Toxics (OPPT), and Center for Computational Toxicology and Exposure (CCTE) — nominated substances for testing. Many of these substances fall outside the already evaluated types or groupings of substances tested for use in the OECD test guidelines, thus allowing for a potential expansion upon the types of substances that can be successfully assessed with these methods.

1.2. AOP for Skin Sensitization with Key Events as Targets of Alternative Method Development

Skin sensitization is an adverse outcome that occurs in two phases; induction of sensitization followed by elicitation of an immune reaction (Kimber et al., 2002). The adverse outcome pathway (AOP) for skin sensitization initiated by covalent binding to proteins has been published by OECD (OECD, 2014) and described by others (MacKay et al., 2013; Maxwell et al., 2014; Strickland et al., 2019) (Figure 1).

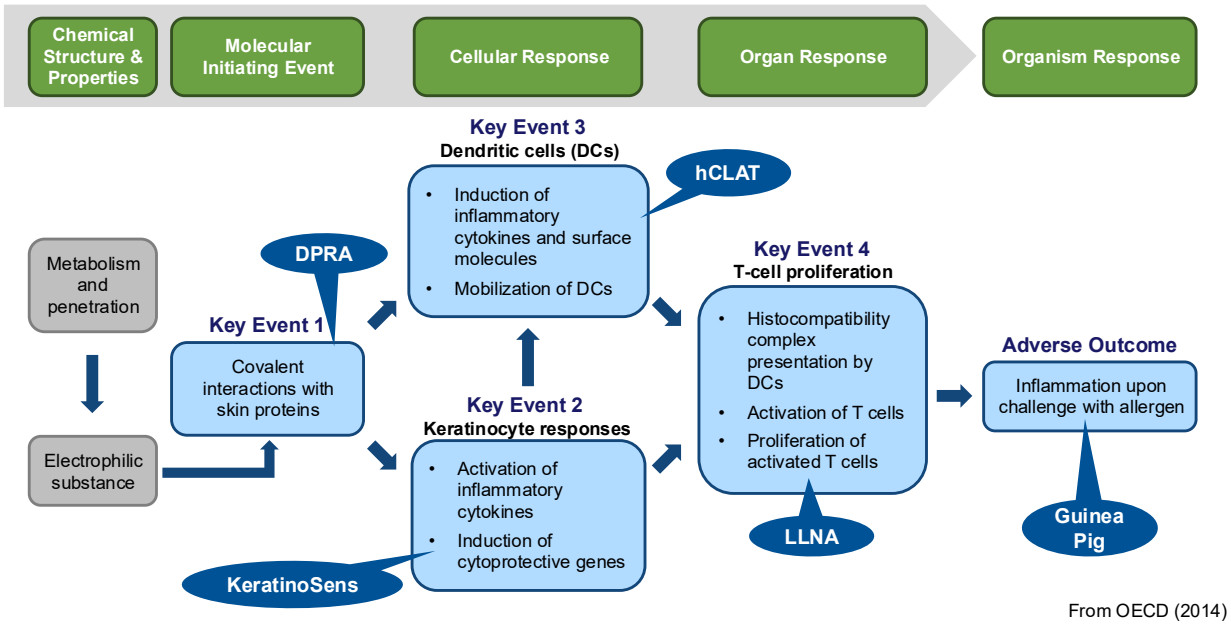
The molecular initiating event, Key Event (KE)1, occurs when a chemical that is either naturally electrophilic or is made electrophilic via auto-oxidation or metabolism penetrates the skin and reacts with lysine or cysteine residues in epidermal proteins, resulting in haptenation through covalent interaction. These peptide/chemical complexes are recognized by immune cells and activate the cascade of events leading to dermal sensitization. The molecular initiating event in KE1 is measured by the in chemico test method DPRA (OECD, 2023b), which measures depletion of synthetic peptides containing lysine or cysteine as test chemicals covalently bind to the synthetic peptides.

KE2 is the initiation of an inflammatory response with induction of inflammatory cytokines and cytoprotective genes including the Nrf2-dependent antioxidant response element (ARE) in keratinocytes. KE2 is addressed by the KeratinoSens assay (OECD, 2023b), which measures luciferase gene induction using the KeratinoSens cell line. This cell line has a stable insertion of the luciferase reporter gene under the control of the ARE element.

KE3 is the activation of dendritic cells (DCs) with induction of inflammatory cytokines and surface molecules and mobilization of dendritic cells. KE3 is addressed by h-CLAT (OECD, 2023c), which measures the cell surface marker expression of CD86 and CD54 on a human monocytic leukemia cell line, THP-1 cells.

KE4 is T cell activation with histocompatibility complexes presented by DCs leading to T cell proliferation, which is typically measured by the in vivo LLNA (OECD, 2010). The adverse outcome is an inflammatory response upon challenge with an allergen and can be assessed using the guinea pig maximization test or the Buehler test (OECD, 2022a).

While each of the three in vitro/in chemico assays evaluated for this project addresses a KE in the AOP, no single in vitro assay is sufficiently predictive to derive a skin sensitization hazard or potency classification for regulatory purposes. DAs combine the results from these methods so that they can be used to derive regulatory classifications.



From OECD (2014)

Figure 1. AOP for Skin Sensitization

1.3. Objectives

The objectives of this study were to: 1) characterize the skin sensitization hazard and potency of substances nominated by ICCVAM agency members using in vitro tests for skin sensitization, 2) compare the hazard classification based on in vitro/in chemico test data to in vivo reference data, 3) use the in vitro/in chemico test data as inputs to DAs for hazard and potency classification, 4) compare the DA classifications to in vivo outcomes, and 5) consider the results with respect to individual agency remits and whether the methods perform well with these potentially difficult-to-test substances.

2. METHODS

2.1. Substances Nominated by Agency Partners for NAM Evaluation

Six ICCVAM agencies or offices within agencies nominated 185 substances. The substance names, Chemical Abstracts Services Registry Numbers (CASRNs; if available), BRT in vitro identification code (BRTIV), lot number, and supplier information for each substance are provided in Tables 1–5. Substances that were nominated by an agency but were not tested in any assays are listed in Table 6 along with the exclusion reason.

Table 1. DTT- and FDA-Nominated Substances

DTT-Nominated Substances

BRTIV Number	CASRN	Substance Name	Lot Number	Supplier
BRTIV-1	5466-77-3	2-Ethylhexyl p-methoxycinnamate	A0293319	Acros Organics via Fisher Scientific
BRTIV-2	109-86-4	2-Methoxyethanol	SHBD1377V	Sigma-Aldrich
BRTIV-3	34885-03-5	4-Methylcyclohexanemethanol	KDY3F	TCI America via MRIGlobal
BRTIV-4[a]	693-13-0	Diisopropylcarbodiimide	09330DR	Sigma-Aldrich
BRTIV-5	107-15-3	Ethylenediamine	015K0613	Sigma-Aldrich
BRTIV-6[b]	54464-57-2	Iso E Super	1-LWJ-148-1	Toronto Research Chemicals
BRTIV-7[c]	4098-71-9	Isophorone diisocyanate	03003PB	Sigma-Aldrich
BRTIV-8	119-36-8	Methyl salicylate	03117LC	Sigma-Aldrich
BRTIV-9	141-32-2	n-Butyl acrylate	06805HO	Sigma-Aldrich
BRTIV-11	15625-89-5	Trimethylolpropane triacrylate	08304EE	Sigma-Aldrich
BRTIV-12	479500-35-1	1-Butyl-1-methylpyrrolidinium	20100610	Promy Chemical Inc.
BRTIV-13	79917-90-1	1-Butyl-3-methylimidazolium chloride	99/787	Solvent Innovation GmbH via MRIGlobal
BRTIV-14	65039-09-0	1-Ethyl-3-methylimidazolium chloride	STBB3624	Sigma-Aldrich via MRIGlobal
BRTIV-15	93-76-5	2,4,5-Trichlorophenoxyacetic acid	048K1648	Sigma-Aldrich
BRTIV-16	95-80-7	2,4-Diaminotoluene	1364547	Sigma-Aldrich
BRTIV-17	149-30-4	2-Mercaptobenzothiazole	12607BD	Sigma-Aldrich
BRTIV-18	97-52-9	2-Methoxy-4-nitroaniline	10142299	Alfa Aesar
BRTIV-19	95-83-0	4-Chloro-o-phenylenediamine	14606ED	Sigma-Aldrich
BRTIV-20	2835-95-2	5-Amino-o-cresol	385913/1	Fluka via Sigma-Aldrich
BRTIV-21	83905-01-5	Azithromycin	8411-60-01 (RTI)	Virginia Commonwealth University
BRTIV-22	81103-11-9	Clarithromycin	8409-116-02	Virginia Commonwealth University
BRTIV-23	538-75-0	Dicyclohexylcarbodiimide	60104-1	Chem-Impex International Inc.
BRTIV-24	97-00-7	Dinitrochlorobenzene	01201DJ	Sigma-Aldrich
BRTIV-25	96-45-7	Ethylene thiourea	03903KC	Sigma-Aldrich
BRTIV-26	76-44-8	Heptachlor	32455-05	Radian International LLC
BRTIV-27	1124-64-7	N-Butyl-pyridinium chloride	20100610	Promy Chemical Inc.
BRTIV-28	120-32-1	o-Benzyl-p-chlorophenol	KM11195	McKesson Chemical
BRTIV-29	95-48-7	o-Cresol	RC-890	Merisol USA LLC via Merichem Company
BRTIV-30	7778-50-9	Potassium dichromate	84798MJ	Sigma-Aldrich
BRTIV-31	87-66-1	Pyrogallol	010326	Aceto Corporation via Battelle
BRTIV-32	6834-92-0	Sodium metasilicate	02415CH	Sigma-Aldrich
BRTIV-33	115-86-6	Triphenyl phosphate	8537-05082012-1	Acros Organics via Fisher Scientific

Table 1 (Continued). DTT- and FDA-Nominated Substances

DTT-Nominated Substances

BRTIV Number	CASRN	Substance Name	Lot Number	Supplier
BRTIV-34	431-03-8	2,3-Butanedione	03798LJ	Sigma-Aldrich
BRTIV-35	1393-63-1	Annatto	5512CD	Sensient Colors LLC
BRTIV-35	1393-63-1	Annatto	202005040021	DDW The Color House
BRTIV-36	140-88-5	Ethyl acrylate	07227MC	Sigma-Aldrich
BRTIV-37	78-84-2	Isobutyraldehyde	SHBF4656V	Sigma-Aldrich
BRTIV-38	3524-68-3	Pentaerythritol triacrylate	1136114	ABCR GmbH & Co.
BRTIV-39	121-54-0	Benzethonium chloride	W0061	Battelle Memorial Institute
BRTIV-40	111-30-8	Glutaraldehyde	025K5003	Sigma-Aldrich
BRTIV-41	1912-24-9	Atrazine	4-XJZ-154-1	Toronto Research Chemicals Inc.
BRTIV-42	2921-88-2	Chlorpyrifos	3-ABY-19-1	Toronto Research Chemicals Inc.
BRTIV-43	542-40-5	Norbixin	9000-8-14	MRIGlobal
BRTIV-44	4170-30-3	Crotonaldehyde	1106903	Sigma-Aldrich
BRTIV-45	75-91-2	Tert-butyl hydroperoxide (70% in H_2O)	59797MJ	Sigma-Aldrich
BRTIV-46	105-08-8	1,4-Cyclohexanedimethanol	08102BD	Sigma-Aldrich
BRTIV-55	6983-79-5	cis-Bixin	24139	Pfaltz & Bauer Inc.
BRTIV-57	86386-73-4	Fluconazole	X4YGD	TCI America
BRTIV-77	3173-72-6	1,5-Naphthalene diisocyanate	4SYNE	TCI America
BRTIV-83	14897-39-3	Rifamycin-SV	18-ANR-147-1	Toronto Research Chemicals
BRTIV-86	98955-27-2	4-Methoxymethylcyclohexanemethanol	13296-9-8	MRIGlobal
BRTIV-87	4331-54-8	4-Methylcyclohexanecarboxylic acid	DBUVA	TCI America
BRTIV-88	34885-03-5	4-Methylcyclohexanemethanol	KDY3F	TCI America
BRTIV-89	NA	Crude MCHM	TP14044373	Eastman Chemical Company
BRTIV-90	498-81-7	Cyclohexanemethanol, alpha, alpha, 4-trimethyl-	H2014	Santa Cruz Biotechnology Inc
BRTIV-93	94-60-0	Dimethyl 1,4-cyclohexanedicarboxylate	02610LH	Sigma-Aldrich
BRTIV-95	51730-94-0	Dipropylene glycol phenyl ether, DiPPH, purified product	200602920-14	DOW Chemical Company
BRTIV-96	NA	Dipropylene glycol phenyl ether, DiPPH, Dowanol commercial product	2I040195K1	DOW Chemical Company
BRTIV-101	51181-40-9	Methyl 4-methylcyclohexanecarboxylate	2-MKM-124-1	Toronto Research Chemicals
BRTIV-103	4169-04-4	Phenoxyisopropanol	FII01	TCI America
BRTIV-105	770-35-4	Propylene glycol phenyl ether (phenoxypropanol)	YP0924	Spectrum Chemical Mfg. Corp.
BRTIV-111	600-14-6	2,3-Pentanedione	MKBB7504	Sigma-Aldrich

Table 1 (Continued). DTT- and FDA-Nominated Substances

DTT-Nominated Substances

BRTIV Number	CASRN	Substance Name	Lot Number	Supplier
BRTIV-115	114651-37-5	Cyclohexanemethanol, 4-((ethenyloxy)methyl)-	09113PAV	Sigma-Aldrich
BRTIV-120	74-94-2	Dimethylamine borane	STBG5064V	Sigma-Aldrich
BRTIV-132	15972-60-8	Alachlor	6425500	Chem Service Inc.

[a] Lot 13016JS from Sigma-Aldrich for BRTIV-4 (diisopropylcarbodiimide) was tested initially but there was not enough material to complete all three in vitro methods. A new lot was retested in all assays to make a prediction. Results obtained from the previous lot are provided in the appropriate tables and appendices.

[b] Lot RAV0276433 from International Flavors & Fragrances Inc. via Virginia Commonwealth University for BRTIV-6 (Iso E Super) was tested initially but there was not enough material to complete all three in vitro methods. A new lot was retested in all assays to make a prediction. Results obtained from the previous lot are provided in the appropriate tables and appendices.

c Lot STBH3457from Sigma-Aldrich for BRTIV-7 (isophorone diisocyanate) was retested in DPRA and KeratinoSens, but not h-CLAT. Results obtained from both lots are provided in the appropriate appendices.

FDA-Nominated Substances

BRTIV Number	CASRN	Substance Name	Lot Number	Supplier
BRTIV-10	102-71-6	Triethanolamine	03421DJ	Sigma-Aldrich
BRT IV-191	79416-27-6	Methyl aminolevulinate hydrochloride	67865	MedChemExpress LLC

Table 2. CPSC-Nominated Substances

BRTIV Number	CASRN	Substance Name	Lot Number	Supplier
BRTIV-50	525-76-8	2-Methyl-4H,3,1-benzoxazin-4-one (Product 2040)	09815JJV	Sigma-Aldrich
BRTIV-52	6358-09-4	Amino-6-chloro-4-nitrophenol	5VHBE	TCI America
BRTIV-56	10026-24-1	Cobalt sulfate heptahydrate	SLBQ9230V	Sigma-Aldrich
BRTIV-59[a]	7786-81-4	Nickel (II) sulfate	MKCC3397	Sigma-Aldrich
BRTIV-64	97-77-8	Tetraethylthiuramdisulfide	027K1529	Sigma-Aldrich
BRTIV-65	97-74-5	Tetramethylthiurammonosulfide	03816EJ	Sigma-Aldrich
BRTIV-68	14324-55-1	Zinc diethyldithiocarbamate	08319ME	Sigma-Aldrich
BRTIV-69	137-30-4	Zinc dimethyldithiocarbamate	416847/1	Sigma-Aldrich
BRTIV-71	7447-39-4	Copper (II) chloride	STBB5791V	Sigma-Aldrich
BRTIV-85	93-16-3	3-Methylisoeugenol	STBD5743V	Sigma-Aldrich
BRTIV-92	109-89-7	Diethylamine	05497HJ	Sigma-Aldrich
BRTIV-97	138-86-3	d-Limonene	DBZAE	TCI America
BRTIV-100	68855-99-2	Litsea cubeba oil	MKAA4731	Sigma-Aldrich
BRTIV-109	98-88-4	Benzoyl chloride	1370010	Sigma-Aldrich
BRTIV-117	584-84-9	Toluene 2,4-diisocyanate	10313PA	Sigma-Aldrich
BRTIV-121	55302-96-0	Methyl 5-hydroxyethylaminophenol	W3LGM	TCI America
BRTIV-134	1210-39-5	Phenyl cinnamic aldehyde	17731JHV	Sigma-Aldrich
BRTIV-136	13878-54-1	Zinc pentamethylenedithiocarbamate	A17X0413	Alfa Chemistry, Protheragen Inc.
BRTIV-140	5406-12-2	p-Methylhydrocinnamaldehyde	S01R	Matrix Scientific
BRTIV-141	176665-09-1	Azalactone C15-C19	WG0667193-170727001	Ark Pharm Inc.
BRTIV-142	104-27-8	Methylanisylidene acetone	WG0060312-170818001	Ark Pharm Inc.
BRTIV-145	26172-55-4	5-Chloro-2-methylisothiazolinone	B77179	Combi-Blocks Inc. via Sigma-Aldrich
BRTIV-156	70-25-7	Methyl-3-nitro-1-nitrosoguanidine	6466900	Chem Service Inc.

[a] BRTIV-59 could not be dissolved in any assay solvent and therefore could not be tested.

Table 3. EPA CCTE-Nominated Substances

BRTIV Number	CASRN	Substance Name	Lot Number	Supplier
BRTIV-47	3344-77-2	12-Bromo-1-decanol	09102PHV	Sigma-Aldrich
BRTIV-49	611-06-3	2,4-Dichloronitrobenzene	06822BD	Sigma-Aldrich
BRTIV-51	2657-25-2	4'-Hydroxychalcone	NAZRH	TCI America
BRTIV-54	10373-78-1	Camphorquinone	09003AQV	Sigma-Aldrich
BRTIV-58	91-68-9	N,N-Diethyl-m-aminophenol	14822HD	Sigma-Aldrich
BRTIV-82	684-93-5	N-Methyl-N-nitrosourea	14-MWC-167-1	Toronto Research Chemicals
BRTIV-98	62-50-0	Ethyl methanesulphonate	DGMLA	TCI America
BRTIV-114	591-87-7	Allyl acetate	01703TD	Sigma-Aldrich
BRTIV-127	112-80-1	Oleic acid	SLBQ3165V	Sigma-Aldrich
BRTIV-135	4230-97-1	Allyl octanoate	B14S05271	BOC Sciences
BRTIV-139	13323-66-5	2-Propen-1-one, 1-(2-hydroxyphenyl)-3-(4-hydroxyphenyl)-	B16S07201	BOC Sciences
BRTIV-144	880-09-1	Dipiperidinomethane	20170828005	Finetech Industry Limited
NA[a]	36727-29-4	3,5,5-Trimethylhexanoyl chloride	06225BGV	Sigma-Aldrich

NA = not applicable.

[a] Substance was not assigned BRTIV numbers for in vitro hypersensitivity testing due to potential hazards of testing as determined by a certified industrial hygienist.

Table 4. EPA OPP-Nominated Substances

BRTIV Number	CASRN	Substance Name	Lot Number	Supplier
BRTIV-75	91465-08-6	Lambda-cyhalothrin	K6 6622D M05	Monsanto Company via Amazon.com
BRTIV-76	709-98-8	Propanil	E8050002	Fine Americas Inc. via Pestrong.com
BRTIV-78	1111-78-0	Ammonium carbamate	YGM8G25008	Syngenta Crop Protection LLC via Do My Own Pest Control
BRTIV-79	71751-41-2	Abamectin	K90991	Bell Laboratories Inc. via Do My Own Pest Control
BRTIV-80	736994-63-1	Cyantraniliprole	WTN-20420	Arborjet via Bartlett Arborist Supply
BRTIV-81	1918-00-9	Dicamba	17K14A2	Cardinal Laboratories Inc. via Crazy Pet Shop
BRTIV-110	109-94-4	Ethyl formate	D058I5J003 06264	Dow AgroSciences LLC via Northwest Crop Protection LLC
BRTIV-119	121-75-5	Malathion	103185	Lawn and Garden Products Inc. via Amazon.com
BRTIV-133	62924-70-3	Flumetralin	17164VA017	Nufarm Americas Inc. via Winfield Solutions LLC
BRTIV-169	38641-94-0	RoundUp Precision Gel Weed and Grass Killer (glyphosate isopropyl amine)	NK25HX0155	Bayer Crop Science LP via Winfield Solutions LLC
BRTIV-170	83657-17-4	Concise Ornamental Plant Growth Regulator (uniconazole-p)	AF7054P01	Arysta Lifescience via Winfield Solutions LLC
BRTIV-171	183675-82-3	Velista Fungicide (penthiopyrad)	MHA8D25-ID1	Syngenta Crop Protection LLC via Winfield Solutions LLC
BRTIV-172	56073-10-0	Final Soft Bait with Lumitrack (brodifacoum)	MHA8F25-FA2	Syngenta Crop Protection LLC via Winfield Solutions LLC
BRTIV-173	138261-41-3	IMA-jet 10 (imidacloprid)	18151AL004	Nufarm Americas Inc. via Winfield Solutions LLC
BRTIV-174	8003-34-7; 51-03-6	Cardinal Pets Flea and Tick Shampoo (pyrethrins; piperonyl butoxide)	GH8D131000	United Phosphorus Inc. via Shoreline Aquatic Solutions
BRTIV-175	2008-39-7; 566191-89-7	GrazonNext HL Herbicide (2,4-D dimethylamine salt; aminopyrilid triisopropanolammonium salt)	CA053192	Ecolab via HP Products
BRTIV-176	10294-56-1	Monterey Garden Phos (phosphorous acid as mono- and di-potassium salts of phosphorous acid)	NL19190A	Reckitt Benckiser LLC via U-Line

Table 4 (Continued). EPA OPP-Nominated Substances

BRTIV Number	CASRN	Substance Name	Lot Number	Supplier
BRTIV-177	103361-09-7	Panther SC Herbicide	A7380260425	Prime Source LLC via Northwest Crop Protection LLC
BRTIV-178	929-06-6; 335104-84-2	DiFlexx Duo	488200	Spartan Chemical Company Inc. via Home Depot
BRTIV-179	361377-29-9	Tepera Fungicide	AO070191	Ecolab Inc. via Kelly Supply Inc.
BRTIV-180	1003318-67-9; 374726-62-2	Orondis Ultra	D548H91810	Dow AgroSciences LLC via Pestrong.com
BRTIV-181	131860-33-8; 119446-68-3	Quadris Top SBX Fungicide	BN L884	Medentech Ltd. via Amazon.com
BRTIV-182	138261-41-3; 57837-19-1; 107534-96-3; 131341-86-1	Sativa IMF Sembolite Max	K6 6622D M05	Monsanto Company via Amazon.com
BRTIV-183	2164-07-0; 85-00-7	Aquastrike	E8050002	Fine Americas Inc. via Pestrong.com
BRTIV-184	25155-30-0; 79-33-4	Antimicrobial Fruit and Vegetable Treatment	YGM8G25008	Syngenta Crop Protection LLC via Do My Own Pest Control
BRTIV-185	68424-85-1	Lysol Professional No Rinse Sanitizer	K90991	Bell Laboratories Inc. via Do My Own Pest Control
BRTIV-186	131860-33-8	Azoxy 2SC Select Fungicide	WTN-20420	Arborjet via Bartlett Arborist Supply
BRTIV-187	7681-52-9	Diffense	17K14A2	Cardinal Laboratories Inc. via Crazy Pet Shop
BRTIV-188	7722-84-1; 67-63-0; 68424-85-1; 32426-11-2; 7173-51-5; 5538-94-3	Drysan Duo	D058I5J003 06264	Dow AgroSciences LLC via Northwest Crop Protection LLC
BRTIV-189	1929-82-4	Instinct HL	103185	Lawn and Garden Products Inc. via Amazon.com
BRTIV-190	2893-78-9	Aquatabs	17164VA017	Nufarm Americas Inc. via Winfield Solutions LLC

Table 5. EPA OPPT-Nominated Substances

BRTIV Number	CASRN	Substance Name	Lot Number	Supplier
BRTIV-48	81-48-1	1-Hydroxy-4-(p-toluidino)anthraquinone	MKBZ0887V	Sigma-Aldrich
BRTIV-53	7783-18-8	Ammonium thiosulfate	KU46M	TCI America
BRTIV-60	92-88-6	p,p'-Biphenol	YWCID	TCI America
BRTIV-61	14024-61-4	Palladium di(4-oxapent-2-en-2-oate)	40920	Sigma-Aldrich
BRTIV-62	13967-50-5	Potassium dicyanoaurate	A0147125	Acros Organics via Fisher Scientific
BRTIV-63	142-31-4	Sodium octyl sulfate	05308KJV	Sigma-Aldrich
BRTIV-66	501-98-4	trans-p-Hydroxycinnamic acid	18705MS	Sigma-Aldrich
BRTIV-67	78-50-2	Tri-n-octylphosphine oxide	10703ED	Sigma-Aldrich
BRTIV-70	14024-63-6	Bis(pentane-2,4-dionato)zinc	S36727	Sigma-Aldrich
BRTIV-72	98-59-9	p-Toluenesulfonyl chloride	54596EJ	Sigma-Aldrich
BRTIV-73	485-47-2	1H-Indene, 1,3(2H)-dione, 2,2-dihydroxy-	05216KF	Sigma-Aldrich
BRTIV-74	1889-67-4	2,3-Dimethyl-2,3-diphenylbutane	BCBS4850V	Sigma-Aldrich
BRTIV-84	25354-97-6	2-Hexyldecanoic acid	02949LH	Sigma-Aldrich
BRTIV-91	1569-69-3	Cyclohexyl mercaptan	09901EW	Sigma-Aldrich
BRTIV-94	624-92-0	Dimethyl disulfide	1-JLW-25-1	Toronto Research Chemicals
BRTIV-99	75-33-2	Isopropyl mercaptan	WG0021183-140807001	Ark Pharm Inc.
BRTIV-102	112-55-0	n-Dodecylmercaptan	14321LH	Sigma-Aldrich
BRTIV-104	28961-43-5	Poly(oxy-1,2-ethanediyl), alpha-hydro-omega-((1-oxo-2-propenyl)oxy)-,ether with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol (3:1)	H64CH3	TCI America
BRTIV-106	111-01-3	Squalane	A0372305	Acros Organics via Fisher Scientific
BRTIV-107	56-23-5	Tetrachloromethane	G00101.4.Inc	IoLiTec
BRTIV-108	78-51-3	Trisbutoxyethyl phosphate	STBF5337V	Sigma-Aldrich
BRTIV-112	3031-66-1	3-Hexyne-2, 5-diol	80630	Sigma-Aldrich
BRTIV-113	5208-93-5	3-Methyl-1-(2,6,6-trimethylcyclohex-1-en-1yl)penta-1,4-dien-3-ol	018K1145	Sigma-Aldrich
BRTIV-116	614-45-9	t-Butyl perbenzoate	2-XJZ-92-1	Toronto Research Chemicals
BRTIV-118	26471-62-5	Toluenediisocyanate	1375434	Sigma-Aldrich
BRTIV-122	2622-14-2	Product containing tricyclohexyl phosphine	H00101.4.Inc	IoLiTec

Table 5 (Continued). EPA OPPT-Nominated Substances

BRTIV Number	CASRN	Substance Name	Lot Number	Supplier
BRTIV-123	3115-68-2	Tetrabutylphosphonium bromide 74-76% in water	G00101.4.Inc	IoLiTec
BRTIV-124	1117-86-8	Capryl glycol	A44296	Combi-Blocks Inc. via Sigma-Aldrich
BRTIV-125	7681-57-4	Disulfurous acid, disodium salt	20170710003	Finetech Industry Limited
BRTIV-126	112-62-9	9-Octadecenoic acid (9Z)-, methyl ester	WG0233981-170814001	Ark Pharm Inc.
BRTIV-128	102691-36-1	2-Cyanoethyl N,N,N',N'-tetraisopropylphosphordiamidite	BCBN5352V	Sigma-Aldrich
BRTIV-129	109-79-5	n-Butylmercaptan	STBD9838V	Sigma-Aldrich
BRTIV-130	258864-54-9	Trihexyl tetradecyl phosphonium chloride	B16ZJ02042	BOC Sciences
BRTIV-131	344774-05-6	Triisobutyl(methyl)phosphonium toluenesulfonamide	B16ZJ04251	BOC Sciences
BRTIV-137	66415-55-2	Aminopropyl vinyl ether	B16ZJ12251	BOC Sciences
BRTIV-138	72676-55-2	1,3,4-Thiadiazole-2(3H)-thione, 5,5'-dithiobis-	B16ZJ06182	BOC Sciences
BRTIV-143	68515-73-1	D-Glucopyranose, oligomeric, decyl octyl glycosides	A16X0318	Alfa Chemistry, Protheragen Inc.
BRTIV-146[a]	84100-23-2	Castor oil, sulfated, sodium salt	B14QT0714	BOC Sciences
BRTIV-147	13557-75-0	2-Propenoic acid, 4-(1,1-dimethylethyl)cyclohexyl ester (4-*tert*-Butylcyclohexyl acrylate)	R25D048	Alfa Aesar via Fisher Scientific
BRTIV-148	10436-39-2	Sodium lauroyl lactylate	30234400	Strem Chemicals Inc. via Fisher Scientific
BRTIV-149	50849-47-3	1,1,2,3-Tetrachloropropene	2GG0360	Spectrum Chemical Mfg. Corp.
BRTIV-150	219770-99-7	2-Hydroxy-5-nonylbenzaldoxime	8H-33768	Gelest Inc.
BRTIV-151	25549-16-0	Ruthenium, dichloro[(phenylthio)methylene]bis(tricyclohexylphosphine)-	14896LJ	Sigma-Aldrich
BRTIV-152	26747-90-0	Triisooctylamine	V28R	Matrix Scientific
BRTIV-153	19168-23-1	1,3-Diazetidine-2,4-dione, 1,3-bis(3-isocyanatomethylphenyl)-	06830CDV	Sigma-Aldrich
BRTIV-154	4706-17-6	Diammonium hexachloropalladate	B16ZJ11302	BOC Sciences
BRTIV-155	61789-40-0[b]	Tris(hydroxypropyl)phosphine	MKBZ0887V	Sigma-Aldrich
BRTIV-157	133-14-2	1-Propanium, 3-amino-N-(caboxymethyl)-n,N-dimethyl-, N-coco acyl derivatives, inner salts	KU46M	TCI America
BRTIV-158	109-99-9	Peroxide, bis(2,4-dichlorobenzoyl)	YWCID	TCI America
BRTIV-159	64265-57-2	Tetrahydrofuran	40920	Sigma-Aldrich
BRTIV-160	7681-57-4	1-Aziridinepropanoic acid, 2-methyl-, 2-ethyl-2-((3-(2-methyl-1-aziridinyl)–1-oxopropoxy)methyl)-1,3-propanediyl	A0147125	Acros Organics via Fisher Scientific

Table 5 (Continued). EPA OPPT-Nominated Substances

BRTIV Number	CASRN	Substance Name	Lot Number	Supplier
NA[c]	303-04-8	2,3-Dichlorohexafluoro-2-butene	06830CDV	Sigma-Aldrich
NA[c]	15520-11-3	Di(tert-butylcyclohexyl)peroxydicarbonate	B16ZJ11302	BOC Sciences

NA = not applicable.

[a] BRTIV-146 could not be dissolved in any assay solvent and therefore could not be tested.

[b] EPA OPPT associated 1-propanium, 3-amino-N-(carboxymethyl)-n,N-dimethyl-, N-coco acyl dervs., inner salts, with CASRN 68139-30-0; however, the substance tested was associated with CASRN 61789-40-0. The EPA CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/) (Williams et al., 2017) associates the tested CASRN with cocamidopropyl betaine, which was 27–34% of the solution tested according to the Certificate of Analysis.

[c] Substances were not assigned BRTIV numbers for in vitro hypersensitivity testing due to potential hazards of testing as determined by a certified industrial hygienist.

Table 6. Nominated Chemicals That Were Not Tested

Nominating Agency	CASRN	Chemical Name	Exclusion Reason
CPSC	7786-81-4	Nickel sulfate (Nickel salts)	Insoluble in assay solvents
EPA CCTE	36727-29-4	3,5,5-Trimethylhexanoyl chloride	Potential hazards associated with testing
EPA OPPT	303-04-8	2,3-Dichlorohexafluoro-2-butene	Potential hazards associated with testing
EPA OPPT	15520-11-3	Di(tert-butylcyclohexyl) peroxydicarbonate	Potential hazards associated with testing
EPA OPPT	68187-76-8	Castor oil, sulfated sodium salt	Insoluble in assay solvents

2.2. In Chemico, In Vitro, and In Silico Data Generated for this Project

Data were generated for this project using in silico, in chemico, and in vitro methods. BRT, the DTT contract laboratory for immunotoxicity testing, tested 180 unique nominated compounds using DPRA, KeratinoSens, and h-CLAT. Due to testing with two different lots of annatto, we will hereafter report that 181 substances were tested. We considered the two different lots of annatto separately because the NAM results were different for the two lots. We considered the two different sources (same lots) of 4-methylcyclohexanemethanol as the same because the NAM results were the same. Sections 2.2.1 to 2.2.3 review the tests performed by BRT. Generation of the in silico data is described in Section 2.3. The comprehensive test reports, which include detailed protocols for the methods and results are provided in Appendix B.

2.2.1. DPRA

Testing was carried out in accordance with OECD Test Guideline 442C (OECD, 2023b). Test substances were evaluated for their reactivity with synthetic peptides containing cysteine or lysine. The concentrations of each peptide in the reaction solution were measured by high pressure liquid chromatography to determine percent depletion over the 24 hr incubation period. Acceptance criteria for assay controls and test substance results were applied as described in OECD Test Guideline 442C (OECD, 2023b).

We classified a test substance as positive if the average lysine or cysteine peptide depletion was greater than 6.38%. If the lysine peptide co-eluted with test chemical, we used the cysteine peptide depletion only to classify substances. Cysteine peptide depletion greater than 13.89% was used to classify substances as positive in the absence of lysine data. Depletion of the peptides was also used to classify the reactivity of each test substance as no or minimal, low, moderate, or high reactivity. Substances classified as no or minimal reactivity were negative in the DPRA, and substances classified in any of the other classes were positive in the DPRA.

The reactivity categories were not applied to mixtures such as pesticide products, as the reactivity categories were not developed for use with mixtures. The results reported for this assay include percent lysine peptide depletion, percent cysteine peptide depletion, mean (of cysteine and lysine) peptide depletion, reactivity category, and positive or negative outcome.

2.2.2. KeratinoSens

Test substances were evaluated for activation of the Keap1-Nrf2-ARE-dependent pathway using the immortalized, human-derived keratinocyte cell line KeratinoSens as described in OECD Test Guideline 442D (OECD, 2022b). These keratinocytes have been transfected with a plasmid containing the luciferase gene with expression under the control of the ARE sequence upstream of the SV40 promoter of the AKR1C2 gene. The level of the increase of luciferase expression is associated with keratinocyte activation and is used to classify test substances as nonsensitizers or sensitizers.

In this assay, the KeratinoSens cells were treated with a range of test substance concentrations (Appendix B). After cell lysing, luciferase activity was determined by measuring the luminescence with a luminometer (Molecular Devices SpectraMax® i3 or i3x and data analysis performed using SoftMax® Pro GxP v 6.5.1 or 7.03, respectively). Cell viability was measured using the MTT assay. Acceptance criteria for the assay controls and test substance results were applied as described in OECD Test Guideline 442D (OECD, 2022b). A test substance was considered positive for skin sensitization when all the following conditions were met:

- Average maximum fold induction of luciferase activity was at least 1.5-fold over the solvent control value.
- Cell viability was greater than 70% at the lowest concentration with induction of luciferase activity at greater than or equal to 1.5-fold.
- The effective concentration at 1.5-fold induction was less than 1000 µM.
- There was a dose-dependent increase in luciferase induction.

The results reported for this assay include effective concentration at 1.5-fold induction, maximum fold luciferase induction, inhibitory concentration at cell viability of 50%, and positive/negative outcome.

2.2.3. h-CLAT

Dendritic cell activation in response to test substance exposure is measured in the h-CLAT using the immortalized human monocytic leukemia cell line THP-1 as a dendritic cell surrogate. The THP-1 cells were treated with a range of concentrations of each test substance in a dose range-finding assay in accordance with OECD Test Guideline 442E (OECD, 2023c).

The concentration needed to produce viability of 75% (CV75) was determined from these results and used to calculate the CV75*1.2 for use as the maximum concentration for the main assay. Activation of DCs was assessed by measuring cell surface expression of the costimulatory molecules, CD86 and CD54, which parallels production of the pro-inflammatory cytokines that induce inflammation. Expression of CD86 or CD54 was determined by flow cytometry (BD Accuri™ C6 and data analysis performed with CFlowPlus v1.0.264.21). Propidium iodide staining was used to concurrently assess cell viability in the same cell population.

Acceptance criteria for assay controls and test substance results were applied as described in OECD Test Guideline 442E (OECD, 2023c). An increase in the relative fluorescence intensity greater than or equal to 150% for CD86 and/or greater than 200% for CD54 expression was

indicative of dendritic cell activation and thus a positive response if cell viability at those concentrations was at least 50%.

The results reported for this assay include effective concentration at 150% CD86 induction (EC150), effective concentration at 200% CD54 induction (EC200), inhibitory concentration at cell viability of 50%, CV75, and positive/negative outcome. The minimum induction threshold (MIT), which is the lowest value of the CD54 EC200 and the CD86 EC150, has been derived from the results reported.

2.3. Generation of In Silico Read-Across Hazard Predictions for Skin Sensitization Hazard

In silico read-across predictions for skin sensitization hazard for the test substance nominations were generated using OECD QSAR Toolbox v4.5, which is freely available software (OECD, 2021). The Simplified Molecular Input Line Entry System (SMILES) specifications of chemical structure and CASRNs for each substance were used as inputs to OECD QSAR Toolbox. SMILES matching the CASRNs were obtained from EPA CompTox Chemicals Dashboard (Williams et al., 2017), or alternatively, in the OECD QSAR Toolbox.

Skin sensitization hazard predictions were made using the automated workflow for "EC3 from LLNA or Skin sensitization from GPMT assays for defined approaches (SS AW for DASS)." The workflow provides a prediction of skin sensitization hazard (positive or negative) as well as an assessment of whether each substance evaluated is covered by the applicability domain of the automated workflow. The applicability domain is based on the training set of 2268 substances used to develop the automated workflow, which have LLNA and/or guinea pig maximization test experimental data.

If the automated workflow could not make a prediction because an ingredient was a salt, the salt was dissociated and the automated workflow was applied to the organic portion of the substance to make a prediction. QSAR Toolbox does not make skin sensitization hazard predictions for inorganic structures or for ingredients with undefined structures (e.g., substances of unknown or variable composition, complex reaction products, or biological materials).

2.4. Physicochemical Properties

Physicochemical properties were determined using the Open (Quantitative) Structure-activity/property relationship App (OPERA) v2.7, a free and open-source/open-data suite of QSAR models providing predictions for physicochemical properties, environmental fate parameters, and toxicity endpoints. OPERA is an ongoing collaboration between NICEATM and EPA CCTE (Mansouri et al., 2018).

OPERA predictions of toxicity and physicochemical properties are available through the Integrated Chemical Environment (ICE; https://ice.ntp.niehs.nih.gov/) and the EPA CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/) or can be downloaded from the NIEHS GitHub repository (https://github.com/NIEHS/OPERA).

2.5. Available Reference Data

Historical animal data were used as reference data; no new animal tests were conducted. LLNA data, which are preferred by many regulatory agencies (Daniel et al., 2018) were obtained from multiple literature sources, the NICEATM LLNA database (https://ntp.niehs.nih.gov/whatwestudy/niceatm/test-method-evaluations/skin-sens/llna/index.html), Chemical Effects in Biological Systems database (CEBS, https://cebs.niehs.nih.gov/cebs/), and the EPA OPP and OPPT. The traditional radioactive LLNA as described in Test Guideline 429 (OECD, 2010) was used for both skin sensitization hazard and potency categorization according to the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS; UN, 2021).

Traditional LLNA data were available for 142 substances. One substance that was nominated by OPPT, palladium di(4-oxapent-2-en-2-oate) (CASRN 14024-61-4, BRTIV-61), was associated with an LLNA range-finding study, data from which were used for hazard classification only.

Modified LLNAs such as ex vivo LLNA, cell count LLNA, and non-radioactive guideline versions were used only for reference skin sensitization hazard classification. Twenty-five substances only had data either from modified LLNAs or from LLNA methods that were not clearly described. Thirteen substances had no LLNA data of any kind, but four of these had either guinea pig data or mouse ear swelling test data. These data were also used for skin sensitization hazard classification only. Thus, nine substances had no animal skin sensitization data at all. Eight of these were nominated by DTT and one was nominated by FDA. Data and source information are provided in Appendix A.

2.6. Human Data and Sources

Human reference data, which were available for 24 substances, came primarily from historical human predictive patch tests — the human maximization test and the human repeat insult patch test — which are performed using healthy human volunteers (Strickland et al., 2023). These data were sourced primarily from ICE at https://ice.ntp.niehs.nih.gov/ (Abedini et al., 2021). We also obtained patch test data on both healthy volunteers and workers or patients from the European Chemicals Agency website for Information on Chemicals (https://echa.europa.eu/information-on-chemicals). Data for three substances were from tests on workers, patients, or previously sensitized subjects rather than human predictive patch test data. Human data for methyl aminolevulinate, nominated by the FDA, came from a drug information package for METVIXIA®.

While data for 19 substances were useful for potency classification, data for five substances were useful for hazard classification only. Data and source information are provided in Appendix A.

2.7. Defined Approaches Used for This Project

2.7.1. 2 out of 3 (2o3; DPRA, KeratinoSens, h-CLAT)

The 2o3 DA (Bauch et al., 2012; Urbisch et al., 2015) incorporated into OECD Guideline 497 (OECD, 2023a) provides a skin sensitization hazard classification. This DA uses the outcomes from the in chemico DPRA (KE1), and the in vitro KeratinoSens (KE2) and h-CLAT assays (KE3) (Figure 2). The first step of data interpretation from the 2o3 assay is to determine whether the first two assays performed yield a concordant hazard classification, regardless of testing order. If a concordant classification is not obtained with two assays, a third assay is conducted. If a substance does not have results for at least two assays, the 2o3 result for that substance is not applicable (NA).

We used the OECD Guideline 497 workflow to identify borderline results for DPRA, KeratinoSens, and h-CLAT (OECD, 2023a). The removal of borderline results improved the performance of the 2o3 during the validation assessment for OECD Guideline 497, but also increased the number of substances with inconclusive results for the 2o3 DA. We used the DASS App (To et al., 2024) to determine 2o3 outcomes both with and without the exclusion of borderline results for DPRA, KeratinoSens, and h-CLAT.

For DPRA, OECD Test Guideline 442C requires only one run per substance for definitive results, therefore, only one test run per substance was available to evaluate for borderline results (OECD, 2023b). However, multiple runs per substance were available for the evaluation of borderline results for KeratinoSens and h-CLAT. KeratinoSens (OECD, 2022b) and h-CLAT (OECD, 2023c) outcomes are based on two concordant runs. When two of the runs produced borderline results, the final outcome of the test method (i.e., KeratinoSens or h-CLAT) was "borderline," but if all three runs had different results (i.e., positive, negative, borderline), the final outcome of the test method was "inconclusive."

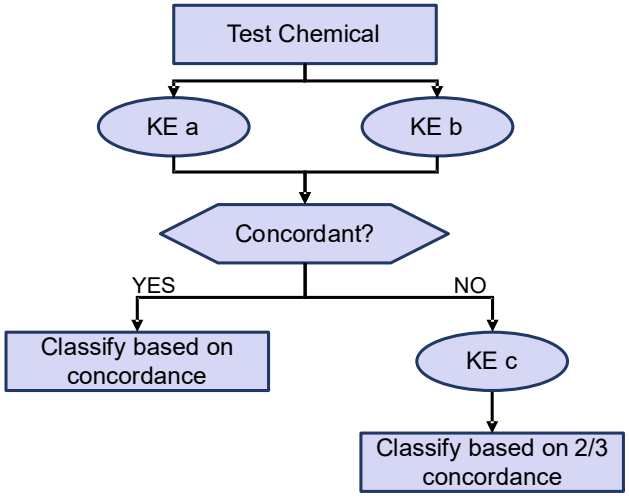


Figure 2. 2o3 DA

2.7.2. Integrated Testing Strategy v2 (ITSv2; h-CLAT, DPRA, OECD Toolbox Prediction)

The ITS DA included in OECD Guideline 497 (OECD, 2023a) was described by Takenouchi et al. (2015). The method was originally described by Nukada et al. (2013), to which Takenouchi et

al. applied an expanded data set. The ITS provides both skin sensitization hazard and GHS potency classification (i.e., 1A, 1B, or Not Classified).

There are two versions of ITS, which differ in the sources used for in silico hazard prediction. We selected ITSv2 over ITSv1 because the ITSv2 uses an in silico hazard prediction from freely available software, OECD QSAR Toolboxv4.5, while ITSv1 requires input from a proprietary source, Derek Nexus v6.1.0. The ITSv2 addresses KE3 of the AOP using h-CLAT and KE1 using DPRA (OECD, 2023a). The ITS uses a scoring system of 0 to 3 for h-CLAT MIT and DPRA peptide depletion results with a score of 0 to 1 for OECD Toolbox hazard (Figure 3).

The scores for the individual inputs are summed and used to predict skin sensitization hazard and GHS potency classification. OECD Guideline 497 includes a workflow for interpreting the total score to consider partial information (e.g., situations in which one input is unavailable) or out-of-domain results for the in silico hazard prediction. In some cases, potency category may not be assigned. We used the DASS App at https://rstudio.niehs.nih.gov/dass/ (To et al., 2024) to determine both hazard and potency classification outcomes for the ITSv2.

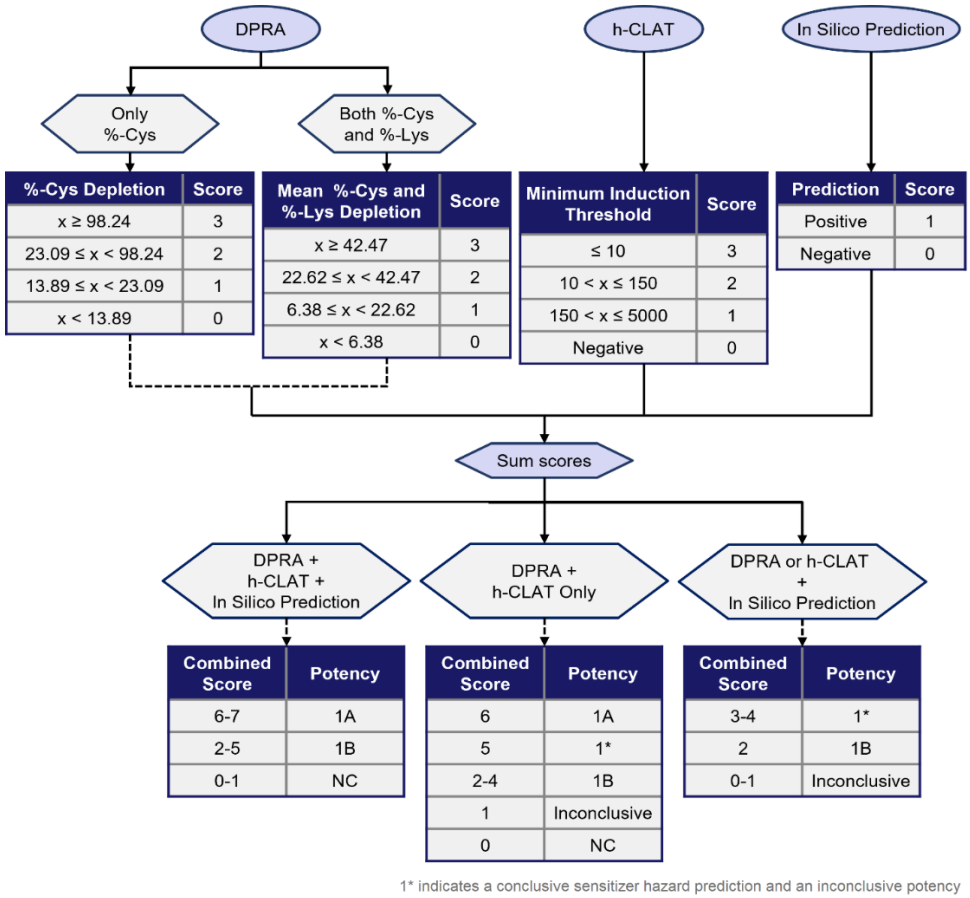


Figure 3. ITSv2 DA

2.7.3. KE3/1 Sequential Testing Strategy (h-CLAT, DPRA)

The KE3/1 STS is accepted by EPA (US EPA, 2018) but is not included in OECD Guideline 497 (OECD, 2023a). The KE3/1 STS was developed by Nukada et al. (2013) and addresses KEs 1 and 3 in the AOP for skin sensitization using the DPRA and h-CLAT, respectively. The KE3/1 STS provides both skin sensitization hazard and GHS potency classification (i.e., 1A, 1B, or Not Classified).

This DA is based on sequential testing beginning with the h-CLAT assay (Figure 4). A test substance producing h-CLAT MIT less than or equal to 10 µg/ml is a "Strong" or GHS Category 1A sensitizer. If the h-CLAT MIT is greater than 10 µg/ml, the substance is a "Weak" or GHS Category 1B sensitizer. If the test substance is negative in the h-CLAT, it is tested using DPRA where a positive result is classified as a "Weak" or GHS Category 1B sensitizer, and a negative result is considered "Not Classified."

We used the DASS App at https://rstudio.niehs.nih.gov/dass/ (To et al., 2024) to determine both hazard and potency classification outcomes for the KE3/1 STS.

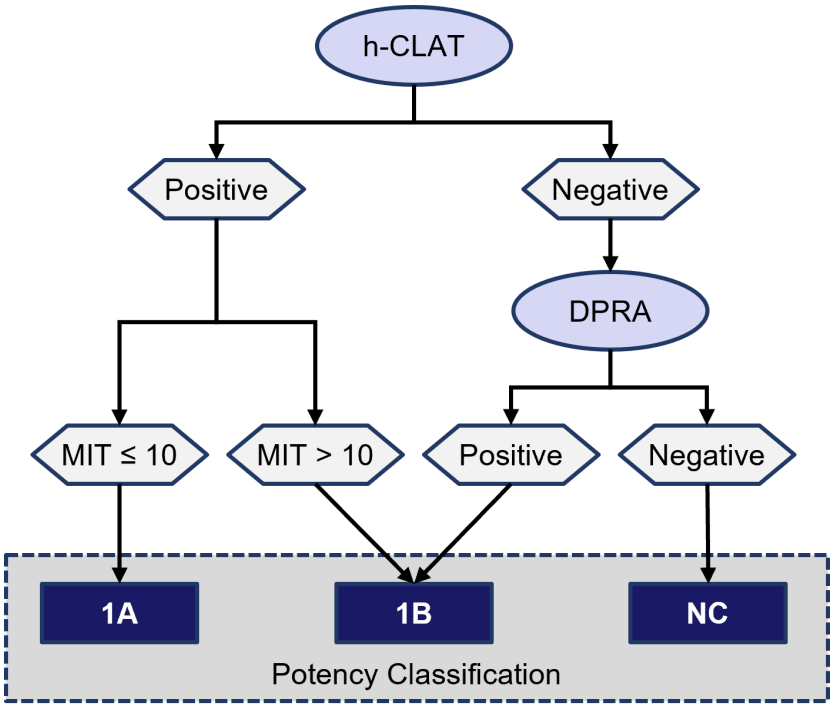


Figure 4. KE 3/1 STS

2.8. Data Analyses

2.8.1. Concordance Analyses

Concordances of the hazard classifications among the individual in vitro methods and DAs were evaluated as well as concordances of these methods with the animal and human data. Concordances of potency classifications among the DAs and between the DAs and animal or human data were also evaluated. The individual in chemico, in vitro, and in silico read-across predictions are not used for potency classification. Concordance analyses are provided using heat

maps, with substances grouped by agency nominator when animal data were included and presented in one group when human data were included.

3. RESULTS

Table 7 summarizes the substances excluded from the analyses, which analyses they were excluded from, and the reason for their exclusion. Primary reasons for exclusion were related to solubility within test systems, but also included interference and chemical instability.

Table 7. Summary of Substances Excluded from Analyses

BRTIV Number	CASRN	Chemical Name	Excluded From	Exclusion Reason
BRTIV-22	81103-11-9	Clarithromycin	DPRA	Insoluble in test solvent
BRTIV-77	3173-72-6	1,5-naphthalene diisocyanate	KeratinoSens, h-CLAT	Insoluble in test solvents
BRTIV-136	13878-54-1	Zinc pentamethylenedithiocarbamate	DPRA	Insoluble in test solvent
BRTIV-109	98-88-4	Benzoyl chloride	KeratinoSens, h-CLAT	Violent reactions and decomposition in the test solvents
BRTIV-58	91-68-9	N,N-Diethyl-m-aminophenol	h-CLAT	Fluoresced at the same wavelength as propidium iodide, which is used for cytotoxicity assessment
BRTIV-61	14024-61-4	Palladium di(4-oxapent-2-en-2-oate)	DPRA	Insoluble in test solvent
BRTIV-153	26747-90-0	1,3-diazetidine-2,4-dione, 1,3-bis(3-isocyanatomethylphenyl)-	DPRA	Insoluble in test solvent
BRTIV-67	78-50-2	Tri-n-octylphosphine oxide	KeratinoSens, h-CLAT	Insoluble in test solvents
BRTIV-74	1889-67-4	2,3-dimethyl-2,3-diphenylbutane	KeratinoSens, h-CLAT	Insoluble in test solvents
BRTIV-122	2622-14-2	Product containing tricyclohexyl phosphine	KeratinoSens, h-CLAT	Insoluble in test solvents

3.1. DTT-Nominated Substances

In all, 63 unique DTT-nominated substances were tested for skin sensitization potential using DPRA, KeratinoSens, and h-CLAT. The hazard and potency classifications based on data from the three assays are summarized in Table 8, along with the OECD Toolbox hazard predictions and the in vivo (animal) hazard predictions. Hazard classifications from the three DAs are also provided, as are potency classifications based on the in vivo animal data and the ITSv2 and KE3/1 DAs. This information is detailed by substance in Table 10.

Two substances that yielded inconclusive results for KeratinoSens because of test method limitations are noted in Table 10.

Twenty-eight (43%) of the 65 substances had concordant hazard classifications among all three in vitro methods. Twenty substances were classified as sensitizers by all three in vitro methods, while eight substances were classified as nonsensitizers by all three in vitro methods (Table 10).

After DPRA, KeratinoSens, and h-CLAT data were evaluated for borderline results, 14-32% of the outcomes were borderline or inconclusive. Numbers of borderline results as well as those

predicted to have positive or negative outcomes are summarized in Table 8. Complete results by substance and method are provided in Table 10. Inconclusive results occur when the test guideline criteria for positive or negative results are not met or when all three runs of a test method had different results (i.e., positive, negative, borderline) during the borderline evaluation.

Fifty-five substances (not counting the two duplicates) had in vivo animal data for hazard classification. Data for eight of these were from modified versions of the LLNA that pre-date the LLNA test guideline, three had data from guinea pig tests, and one had data from the mouse ear-swelling test. The modified LLNAs, guinea pig tests, and mouse ear swelling test were used for in vivo hazard classification but not for in vivo GHS potency classification.

For the 2o3 DA, even before the evaluation of borderline results, two substances had inconclusive or NA hazard or potency classifications because the test substance was either not tested in or was inconclusive in at least one of the in vitro methods. These are noted in Table 10. After the evaluation of borderlines, 28 substances had inconclusive or NA results.

For the hazard outcomes of the ITSv2 DA, eight substances had inconclusive calls due to missing in silico results which produced a total ITSv2 DA score of 1. Only 1,5-naphthalene diisocyanate had an NA hazard outcome for the KE3/1 STS because it was not tested in h-CLAT.

Forty-three substances (not counting the two duplicates) had adequate in vivo animal data to derive a potency classification (summarized in Table 8; detailed in Table 10). Of these 43, 35 substances also had ITSv2 potency classifications for comparison, and 42 substances had KE3/1 STS potency classifications. Overall, 52 substances had ITSv2 potency classifications, and 62 substances had KE3/1 STS potency classifications.

Concordances of skin sensitization hazard classifications are summarized in Figure 5. Figure 5 includes 64 substances because it includes both lots of annatto as separate entries since they had different KeratinoSens results. However, the two different sources of 4-methylcyclohexanemethanol were treated as one entry because the in vitro results were the same. Concordances of potency classifications are summarized in Figure 6.

Regardless of whether borderline results were eliminated or not, the same pattern emerged. Concordance was higher among the classifications based on in vitro/in chemico data than between these and classifications based on in vivo animal ("LLNA" in the Figure 5B) data. The highest concordances were among the DAs. The elimination of borderline results increased the concordance of the individual test methods and the 2o3 DA with the LLNA and increased the concordance of the in vitro/in chemico methods with each other. However, the number of results available for comparison was lower due to the elimination of borderline or inconclusive results. DPRA was the individual method with the highest concordance with the LLNA and, once borderline and inconclusive results were removed, the 2o3 DA was the DA with the highest concordance with the LLNA.

For potency, the concordance of the ITSv2 DA with the KE 3/1 STS DA was higher than the concordance of either DA with the LLNA.

Table 8. Summary of DTT-Nominated Substance Results

Method	Unique Number Evaluated (Total Evaluated)[a]	Initial Number of INC or NA	Final Number of BL, INC, or NA[b]	Number Positive	Number Negative
DPRA Hazard	63 (64)	0	17	27	20
KS Hazard	63 (64)	2	9	41	14
h-CLAT Hazard	63 (64)	0	21	36	7
QSAR TBv4.5 Hazard	63 (65)	—	10	41 (40) [c]	14 (6) [c]
In Vivo GHS Hazard	57	—	0	31	26
In Vivo GHS Potency	43 (45)	—	12	27 (1A:12, 1B:15)	18
2o3 Hazard	63 (64)	2	28	31	6
ITSv2 Hazard	63 (65)	—	8	45	12
ITSv2 GHS Potency	63 (65)	—	11	42 (1A:8, 1B:34)	12
STS Hazard	63 (65)	—	1	54	3
STS GHS Potency	63 (65)	—	1	54 (1A:9, 1B:45)	10

Dashes (—) indicate the parameter is not relevant for that test method. Abbreviations: BL = borderline; INC = inconclusive; NA = not applicable or available.

Colors indicate the different types of assays/reference data: Yellow = in chemico/in vitro, Blue = in silico, Green = reference data, Pink = DAs.

[a] Samples of annatto (CASRN 1393-63-1, BRTIV-35) were tested from two different lot numbers; samples of 4-methylcyclohexanemethanol (CASRN 34885-03-5, BRTIV-3), were tested from two sources with the same lot number.

[b] Classification after the borderline evaluation of the individual test methods (DPRA, KeratinoSens, and h-CLAT).

[c] Number in parentheses = number of in-domain predictions.

Table 9. DTT-Nominated Substances Concordant in All In Vitro Assays

Nonsensitizers, by all in vitro methods (8)

BRTIV Number	CASRN	Chemical Name
BRTIV-2	109-86-4	2-Methoxyethanol
BRTIV-8	119-36-8	Methyl salicylate
BRTIV-14	65039-09-0	1-Ethyl-3-methylimidazolium chloride
BRTIV-15	93-76-5	2,4,5-Trichlorophenoxyacetic acid
BRTIV-25	96-45-7	Ethylene thiourea
BRTIV-35, lot 5512CD	1393-63-1	Annatto
BRTIV-57	86386-73-4	Fluconazole
BRTIV-101	51181-40-9	Methyl 4-methylcyclohexanecarboxylate

Sensitizers, by all in vitro methods (20)

BRTIV Number	CASRN	Chemical Name
BRTIV-4	693-13-0	Diisopropylcarbodiimide
BRTIV-5	107-15-3	Ethylenediamine
BRTIV-11	15625-89-5	Trimethylolpropane triacrylate
BRTIV-16	95-80-7	2,4-Diaminotoluene
BRTIV-17	149-30-4	2-Mercaptobenzothiazole
BRTIV-20	2835-95-2	5-Amino-o-cresol
BRTIV-21	83905-01-5	Azithromycin
BRTIV-23	538-75-0	Dicyclohexylcarbodiimide
BRTIV-24	97-00-7	Dinitrochlorobenzene
BRTIV-34	431-03-8	2,3-Butanedione
BRTIV-36	140-88-5	Ethyl acrylate
BRTIV-37	78-84-2	Isobutyraldehyde
BRTIV-38	3524-68-3	Pentaerythritol triacrylate
BRTIV-40	111-30-8	Glutaraldehyde
BRTIV-42	2921-88-2	Chlorpyrifos
BRTIV-43	542-40-5	Norbixin
BRTIV-44	4170-30-3	Crotonaldehyde
BRTIV-45	75-91-2	tert-Butyl hydroperoxide (70% in H_2O)
BRTIV-55	6983-79-5	cis-Bixin
BRTIV-111	600-14-6	2,3-Pentanedione

Table 10. DTT- and FDA-Nominated Substance Results

DTT-Nominated Substance Results

BRTIV Number	DPRA Hazard	KS Hazard	h-CLAT Hazard	QSAR TBv4.5 Hazard	In Vivo Hazard	In Vivo GHS Potency	2o3 Hazard	ITSv2 Hazard	ITSv2 GHS Potency	STS Hazard	STS GHS Potency
BRTIV-1	0	1	INC / 0	1	0	NC	INC / 0	0	NC	0	NC
BRTIV-2	0	0	0	INC	0	NA	0	0	NC	0	NC
BRTIV-3	0	1	1	1	0	NC	1	1	1B	1	1B
BRTIV-4	1	1	1	INC	1	1B	INC / 1	1	1B	1	1B
BRTIV-5	BL / 1	1	INC / 1	1	1	1B	1	1	1B	1	1B
BRTIV-6	BL/ 0	0	1	1	1	1B	INC / 0	1	1B	1	1B
BRTIV-7	1	0	BL / 0	1	1	1A	INC / 0	1	1B	1	1B
BRTIV-8	0	0	0	0	0	NC	0	0	NC	0	NC
BRTIV-9	1	1	0	1	1	1B	1	1	1B	1	1B
BRTIV-11	1	1	1	1	1	1A	1	1	1A	1	1A
BRTIV-12	0	1	BL / 1	NA	0	NC	INC / 1	INC	INC	1	1B
BRTIV-13	BL / 0	INC / 0	1	NA	0	NC	INC / 0	INC	INC	1	1B
BRTIV-14	0	0	BL / 0	NA	0	NC	0	0	NC	0	NC
BRTIV-15	0	0	INC / 0	0	1	1B	0	0	NC	0	NC
BRTIV-16	1	1	1	1	1	1B	1	1	1B	1	1B
BRTIV-17	1	1	1	1	1	1A	1	1	1B	1	1B
BRTIV-18[a]	BL / 1	INC / 0	1	1	0	NC	INC / 1	1	1B	1	1B
BRTIV-19	1	0	BL / 1	1	1	NA	INC / 1	1	1B	1	1A
BRTIV-20	1	1	1	1	1	1B	1	1	1B	1	1B
BRTIV-21	1	1	1	1	1	1B	1	1	1B	1	1B
BRTIV-22	NT	1	1	1	0	NC	1	1	INC	1	1B
BRTIV-23	1	1	1	INC	1	1A	1	1	INC	1	1A
BRTIV-24	1	1	1	1	1	1A	1	1	1A	1	1A
BRTIV-25	0	BL / 0	BL / 0	0	1	NA	INC / 0	0	NC	0	NC
BRTIV-26	BL / 0	1	1	1	0	NC	1	1	1B	1	1A
BRTIV-27	0	1	1	NA	0	NC	1	INC	INC	1	1B
BRTIV-28	1	BL / 0	1	1	1	NA	1	1	1B	1	1B
BRTIV-29	BL / 0	0	1	INC	0	NC	INC / 0	1	1B	1	1B
BRTIV-30	0	1	1	NA	1	1A	1	1	1B	1	1A
BRTIV-31	BL / 1	0	1	1	1	1A	INC / 1	1	1B	1	1B

Table 10 (Continued). DTT- and FDA-Nominated Substance Results

DTT-Nominated Substance Results, continued

BRTIV Number	DPRA Hazard	KS Hazard	h-CLAT Hazard	QSAR TBv4.5 Hazard	In Vivo Hazard	In Vivo GHS Potency	2o3 Hazard	ITSv2 Hazard	ITSv2 GHS Potency	STS Hazard	STS GHS Potency
BRTIV-32	1	INC / 0	1	NA	0	NC	INC / 1	1	1B	1	1B
BRTIV-33	0	1	1	INC	1	NA	1	1	1B	1	1B
BRTIV-34	1	1	1	1	1	1B	1	1	1A	1	1B
BRTIV-35[b]	0	INC / 0	BL / 0	1	1	1B	INC / 0	0	NC	0	NC
BRTIV-35[c]	1	1	BL / 0	1	1	1B	INC / 1	1	1B	1	1B
BRTIV-36	1	1	1	1	1	1B	1	1	1B	1	1B
BRTIV-37	1	1	INC / 1	1	0	NA	1	1	1B	1	1B
BRTIV-38	1	1	1	1	1	1A	1	1	1A	1	1A
BRTIV-39	0	1	BL / 1	NA	0	NA	INC / 1	1	1B	1	1A
BRTIV-40	1	1	1	1	1	1A	1	1	1A	1	1A
BRTIV-41[a,d]	0	INC	INC / 1	1	0	NC	INC	1	1B	1	1B
BRTIV-42	1	1	1	1	0	NC	1	1	1B	1	1B
BRTIV-43	1	1	1	1	0	NC	1	1	1A	1	1B
BRTIV-44	1	1	1	1	0	NA	1	1	1A	1	1B
BRTIV-45	1	1	1	1	0	NC	1	1	1A	1	1B
BRTIV-46	BL / 1	1	0	1	0	NA	INC / 1	1	1B	1	1B
BRTIV-55	1	1	INC / 1	1	1	1A	1	1	1B	1	1B
BRTIV-57	BL / 0	0	INC / 0	1	0	NA	INC / 0	0	NC	0	NC
BRTIV-77[d]	1	NT	NT	1	1	1A	NA	1	INC	NA	NA
BRTIV-83	1	BL / 0	0	1	0	NC	INC / 0	1	1B	1	1B
BRTIV-86	0	BL / 0	BL / 1	1	ND	ND	INC / 0	1	1B	1	1B
BRTIV-87	0	INC / 0	1	0	ND	ND	INC / 0	0	NC	1	1B
BRTIV-88	0	1	INC / 1	1	0	NC	INC / 1	1	1B	1	1B
BRTIV-89	BL / 0	1	1	NA	1	1B	1	INC	INC	1	1B
BRTIV-90	0	1	1	INC	ND	ND	1	1	1B	1	1B
BRTIV-93	1	0	0	0	0	NA	0	0	NC	1	1B
BRTIV-95	BL / 0	1	BL / 0	0	ND	ND	INC / 0	0	NC	0	NC
BRTIV-96	0	1	BL / 1	NA	ND	ND	INC / 1	INC	INC	1	1B
BRTIV-101	0	0	0	INC	ND	ND	0	0	NC	0	NC
BRTIV-103	0	0	1	INC	ND	ND	INC / 0	INC	INC	1	1B

Table 10 (Continued). DTT- and FDA-Nominated Substance Results

DTT-Nominated Substance Results, continued

BRTIV Number	DPRA Hazard	KS Hazard	h-CLAT Hazard	QSAR TBv4.5 Hazard	In Vivo Hazard	In Vivo GHS Potency	2o3 Hazard	ITSv2 Hazard	ITSv2 GHS Potency	STS Hazard	STS GHS Potency
BRTIV-105	BL / 0	1	INC / 1	INC	0	NA	INC / 1	INC	INC	1	1B
BRTIV-111	1	1	1	1	1	1B	1	1	1B	1	1B
BRTIV-115	BL / 0	1	1	1	ND	ND	1	1	1B	1	1B
BRTIV-120	0	1	BL / 1	NA	1	1B	INC / 1	INC	INC	1	1B
BRTIV-132	1	1	0	1	1	1A	1	1	1B	1	1B

Abbreviations: BL = borderline; INC = inconclusive; NA = not applicable or available; NC = not classified; ND = no data; NT = not tested; 1 = positive; 0 = negative.
Colors indicate the different types of assays/reference data: Yellow = in chemico/in vitro, Blue = in silico, Green = reference data, Pink = DAs.
For DPRA, KS, and h-CLAT hazard results, if a BL result differed from the non-BL result, both are listed, separated by a slash. BL is listed first.

[a] KeratinoSens results were inconclusive because, while there was no positive response observed, test substance solubility was too low to allow testing at concentrations specified in the test guideline.

[b] Lot 5512CD.

[c] Lot 20200504002.

[d] Hazard or potency classification was inconclusive or NA because the test substance was either not tested in or was inconclusive in at least one of the three in vitro methods.

FDA-Nominated Substance Results

BRTIV Number	DPRA Hazard	KS Hazard	h-CLAT Hazard	QSAR TBv4.5 Hazard	In Vivo Hazard	In Vivo GHS Potency	2o3 Hazard	ITSv2 Hazard	ITSv2 GHS Potency	STS Hazard	STS GHS Potency
BRTIV-10	0	INC / 0	1	0	0	NC	INC / 0	0	NC	1	1B
BRTIV-191	1	1	0	NA	ND	ND	1	1	1B	1	1B

Abbreviations: INC = inconclusive; NA = not applicable or available; NC = not classified; ND = no data; 1 = positive; 0 = negative.
Colors indicate the different types of assays/reference data: Yellow = in chemico/in vitro, Blue = in silico, Green = reference data, Pink = DAs.

3.1.1. Concordance of NAMs and Animal Data for DTT-Nominated Substances

A. **B.**

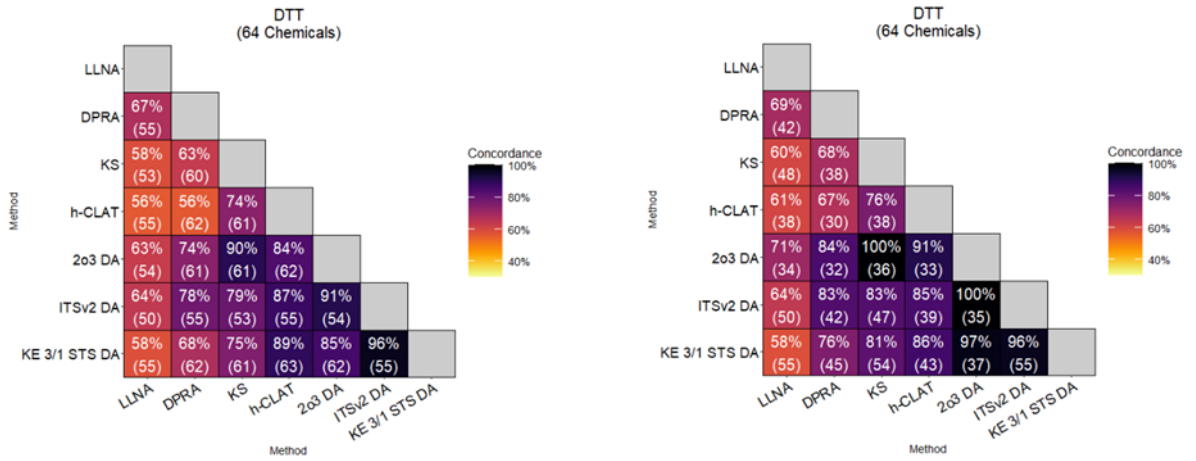


Figure 5. DTT Concordance of Skin Sensitization Hazard Classifications for NAMs and LLNA.
5A shows concordance of all applicable results while 5B shows the concordance following application of borderline exclusion criteria to individual assays and the 2o3 DA. Inconclusive results are not shown.

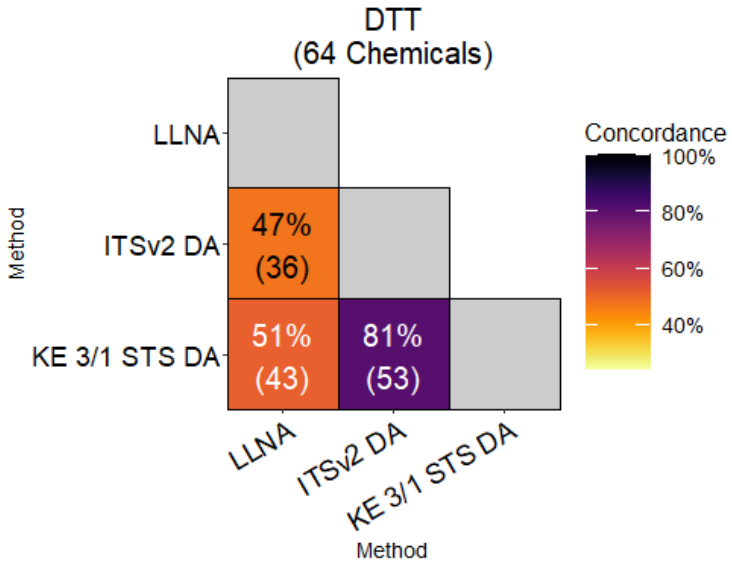


Figure 6. DTT Concordance of Skin Sensitization Potency Classifications for DAs and LLNA.

3.2. FDA-Nominated Substances

The hazard classifications for DPRA, KeratinoSens, and h-CLAT for the two substances nominated by FDA are provided in Table 10 along with the OECD Toolbox hazard and the in

vivo (animal) hazard. Hazard classifications from the three DAs are also provided, as are potency classifications based on the in vivo animal data and the ITSv2 and KE3/1 STS DAs.

Neither substance had concordant hazard classifications based on data from the three in vitro methods. BRTIV-10, triethanolamine (CASRN 102-71-6), was classified as a nonsensitizer by DPRA, KeratinoSens, OECD Toolbox, in vivo animal data, and ITSv2. It was classified as a sensitizer by h-CLAT and as a GHS 1B skin sensitizer by KE3/1 STS. When the data were analyzed for borderline results, the KeratinoSens result for triethanolamine was inconclusive, and thus the 2o3 DA was as well.

BRTIV-191, methyl aminolevulinate hydrochloride (CASRN 79416-27-6), was classified as a sensitizer by the DPRA, KeratinoSens, and all three DAs; h-CLAT classified it as a nonsensitizer. OECD Toolbox did not produce a hazard classification and there were no animal in vivo data, however, human data indicate that it is a sensitizer (see Appendix A). Both DAs that provide potency information classified it as a 1B sensitizer; however, there was no in vivo potency information available for comparison.

3.3. CPSC-Nominated Substances

In all, 22 unique CPSC-nominated substances were tested for skin sensitization potential using DPRA, KeratinoSens, and h-CLAT. The hazard and potency classifications for the three assays are summarized in Table 12, along with the OECD Toolbox hazard predictions and the in vivo (animal) hazard predictions. Hazard classifications from the three DAs are also provided, as are potency classifications based on the in vivo animal data and the ITSv2 and KE3/1 DAs. This information is detailed by substance in Table 13. The four substances that produced inconclusive results from the in vitro methods because of test method limitations are also noted in Table 13.

Ten substances were classified as sensitizers by all three in vitro methods (Table 11). No substances were classified as nonsensitizers by all three methods.

After DPRA, KeratinoSens, and h-CLAT data were evaluated for borderline results in the 2o3 DA, up to one-third of the outcomes were borderline or inconclusive. Numbers of borderline results as well as those predicted to have positive or negative outcomes are summarized in Table 12. Complete results by BRTIV number and method are provided in Table 13. Three substances for which OECD QSAR Toolbox v4.5 hazard predictions could not be obtained are marked "NA" in Table 13, with details provided in the footnotes. Predictions that were outside the QSAR Toolbox applicability domain are marked with "INC" in Table 13.

All 22 substances had in vivo animal data for hazard classification; data for six of these were from modified ex vivo versions of the LLNA that were used for in vivo hazard classification but not for in vivo GHS potency classification. All substances were classified as sensitizers.

For the 2o3 DA, even before the evaluation of borderline results, three substances had inconclusive or NA hazard or potency classifications because the test substance was either not tested in or was inconclusive in at least one of the in vitro methods. These are noted in Table 13, with Table 7 providing substance exclusion reasons. After the evaluation of borderlines, the same three substances had inconclusive or NA results because the 2o3 DA did not provide a conclusive outcome.

For the hazard outcomes of the ITSv2 DA, four substances had inconclusive or NA hazard or potency classifications because the test substance was either not tested in or was inconclusive in at least one of the in vitro methods. These substances are noted in Table 13, but see Table 7 for additional exclusion details. See Figure 3 for the details of how this DA assigns an "INC" outcome.

Summarized concordances of skin sensitization hazard classifications are shown in

A. **B.**

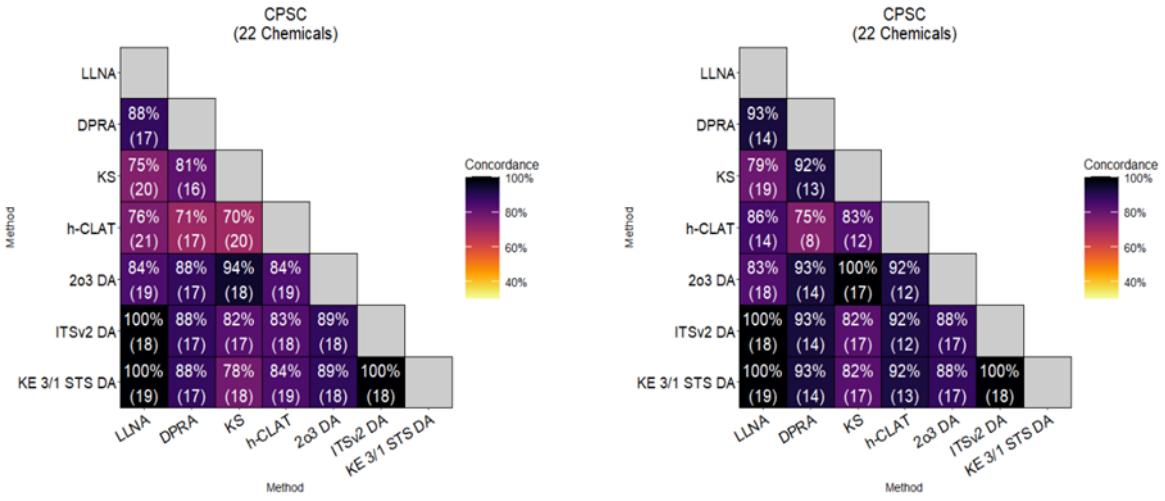


Figure 7, and concordances of potency classifications are shown in Figure 8. Regardless of whether borderline results are eliminated or not, a similar pattern emerged: classifications based on DPRA, KeratinoSens, and h-CLAT have moderate concordance with other methods (70% to 81%) while concordance among DAs was 89% to 100%.

A. **B.**

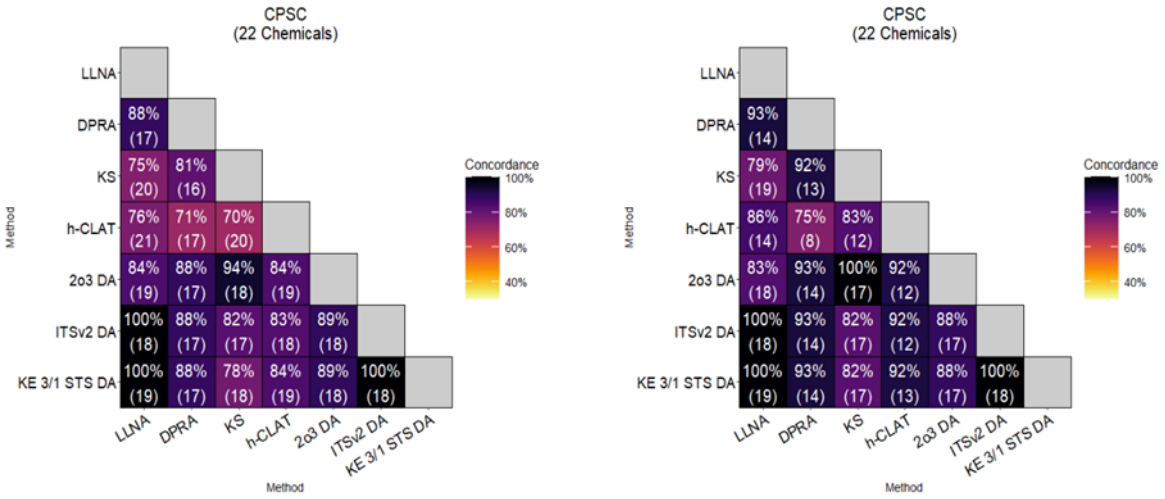


Figure 7A also shows that the concordance of the DA hazard classifications with in vivo LLNA classifications were typically higher (100%) than the concordance of classifications based on in vitro data with LLNA (75% to 88%). However, DPRA had the highest concordance with LLNA at 88%. This pattern remained after borderline results were removed, although overall concordance increased with these adjustments.

For potency, the concordance of the ITSv2 DA with the KE 3/1 STS DA (81%) was higher than the concordance of either DA with the LLNA (54% to 55%).

Table 11. CPSC-Nominated Substances Concordant in All In Vitro Assays
Nonsensitizers (by all in vitro methods) (0)
Sensitizers (by all in vitro methods) (10)

BRTIV Number	CASRN	Chemical Name
BRTIV-56	10026-24-1	Cobalt sulfate heptahydrate
BRTIV-64	97-77-8	Tetraethylthiuramdisulfide
BRTIV-69	137-30-4	Zinc dimethyldithiocarbamate
BRTIV-71	7447-39-4	Copper (II) chloride
BRTIV-100	68855-99-2	Litsea cubeba oil
BRTIV-121	55302-96-0	Methyl 5-hydroxyethylaminophenol
BRTIV-134	1210-39-5	Phenyl cinnamic aldehyde
BRTIV-140	5406-12-2	p-Methylhydrocinnamaldehyde
BRTIV-142	104-27-8	Methylanisylidene acetone
BRTIV-145	26172-55-4	5-Chloro-2-methylisothiazolinone

Table 12. Summary of CPSC-Nominated Substance Results

Method	Unique Number Evaluated[a]	Initial Number of INC or NA	Final Number of BL, INC, or NA[b]	Number Positive	Number Negative
DPRA Hazard	20	3	6	13	1
KS Hazard	21	1	2	15	4
h-CLAT Hazard	21	1	7	12	2
QSAR TBv4.5 Hazard	22	—	3	16 (16)[c]	3 (0)[c]
In Vivo GHS Hazard	22	—	0	22	0
In Vivo GHS Potency	22	—	6	16 (1A:9, 1B:7)	0
2o3 Hazard	22	3	4	15	3
ITSv2 GHS Potency	22	—	6	16 (1A:3, 1B:13)	0
ITSv2 Hazard	22	—	4	18	0
STS Hazard	22	—	3	19	0
STS GHS Potency	22	—	3	19 (1A:5, 1B:14)	0

Dashes (—) indicate the parameter is not relevant for that test method. Abbreviations: BL = borderline; INC = inconclusive; NA = not applicable or available.

Colors indicate the different types of assays/reference data: Yellow = in chemico/in vitro, Blue = in silico, Green = reference data, Pink = DAs.

[a] Two substances, zinc pentamethylenedithiocarbamate (CASRN 13878-54-1, BRTIV-136) and azalactone C15-C19 (CASRN 176665-09-1, BRTIV-141), were not tested in DPRA because they were insoluble in the DPRA solvents. One substance, benzoyl chloride (CASRN 98-88-4, BRTIV-109), was not tested in KeratinoSens or h-CLAT due to a violent reaction with the assay solvent and decomposition in water.

[b] After borderline exclusion of the individual test methods (DPRA, KeratinoSens and h-CLAT).

[c] Number in parentheses = number of in-domain predictions.

Table 13. CPSC Selected Substance Results

BRTIV Number	DPRA Hazard	KS Hazard	h-CLAT Hazard	QSAR TBv4.5 Hazard	In Vivo Hazard	In Vivo GHS Potency	2o3 Hazard	ITSv2 Hazard	ITSv2 GHS Potency	STS Hazard	STS GHS Potency
BRTIV-50[a,b]	INC	1	BL / 0	1	1	1A	INC	INC	INC	NA	NA
BRTIV-52[a]	INC	1	1	1	1	1B	1	1	1B	1	1B
BRTIV-56[c]	1	1	1	NA	1	1A	1	1	1B	1	1B
BRTIV-64	1	1	1	1	1	NA	1	1	1A	1	1A
BRTIV-65	1	1	BL / 0	INC	1	NA	1	1	1B	1	1B
BRTIV-68	BL / 1	0	1	INC	1	NA	INC / 1	1	1B	1	1A
BRTIV-69	1	1	1	1	1	NA	1	1	1A	1	1A
BRTIV-71[c]	1	1	1	NA	1	1A	1	1	INC	1	1B
BRTIV-85	BL / 0	1	1	1	1	1B	1	1	1B	1	1B
BRTIV-92	0	0	1	1	1	NA	0	1	1B	1	1B
BRTIV-97[d]	1	INC	1	1	1	1B	1	1	1B	1	1B
BRTIV-100[e]	1	1	BL / 1	NA	1	1B	1	1	INC	1	1B
BRTIV-109[a,b]	INC	NT	NT	1	1	1A	NA	NA	NA	NA	NA
BRTIV-117	1	0	0	1	1	1A	0	1	1B	1	1B
BRTIV-121	1	1	INC / 1	1	1	1A	1	1	1B	1	1B
BRTIV-134	1	1	1	1	1	1A	1	1	1B	1	1A
BRTIV-136[b]	NT	BL / 0	1	INC	1	NA	INC	NA	NA	1	1A
BRTIV-140	BL / 1	1	1	1	1	1B	1	1	1B	1	1B
BRTIV-141[b]	NT	0	0	1	1	1B	0	INC	INC	NA	NA
BRTIV-142	1	1	INC / 1	1	1	1B	1	1	1B	1	1B
BRTIV-145	1	1	BL / 1	1	1	1A	1	1	1A	1	1B
BRTIV-156	1	1	BL / 0	1	1	1A	1	1	1B	1	1B

Abbreviations: BL = borderline; INC = inconclusive; NA = not applicable or available; NT = not tested; 1 = positive; 0 = negative.

Colors indicate the different types of assays/reference data: Yellow = in chemico/in vitro, Blue = in silico, Green = reference data, Pink = DAs.

For DPRA, KS, and h-CLAT hazard results, if a BL result differed from the non-BL result, both are listed, separated by a slash. BL is listed first.

[a] DPRA results were inconclusive because the test substance co-eluted with the cysteine peptide.

[b] Classification was inconclusive or NA for hazard or potency because the test substance was either not tested in or was inconclusive in at least one of the three in vitro methods.

[c] Inorganic compound.

[d] KeratinoSens results were inconclusive because, while there was no positive response observed, test substance solubility was too low to allow testing at concentrations specified in the test guideline.

[e] Mixture with an undefined structure.

3.3.1. Concordance of NAMs and Animal Data for CPSC-Nominated Substances

A. **B.**

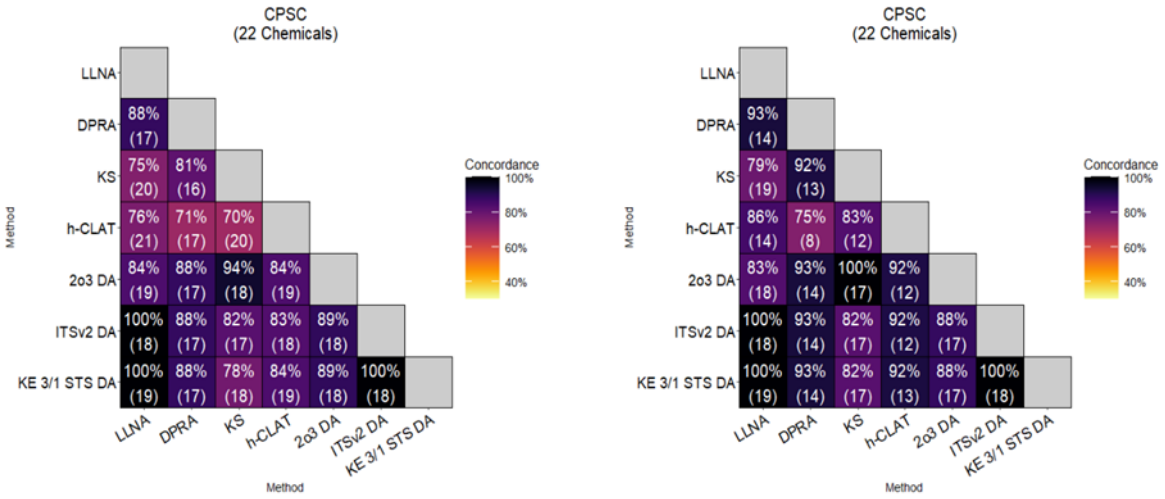


Figure 7. CPSC Concordance of Skin Sensitization Hazard Classifications for NAMs and LLNA.

7A shows concordance of all applicable results while 7B shows the concordance following application of borderline exclusion criteria to individual assays and the 2o3 DA. Inconclusive results are not shown.

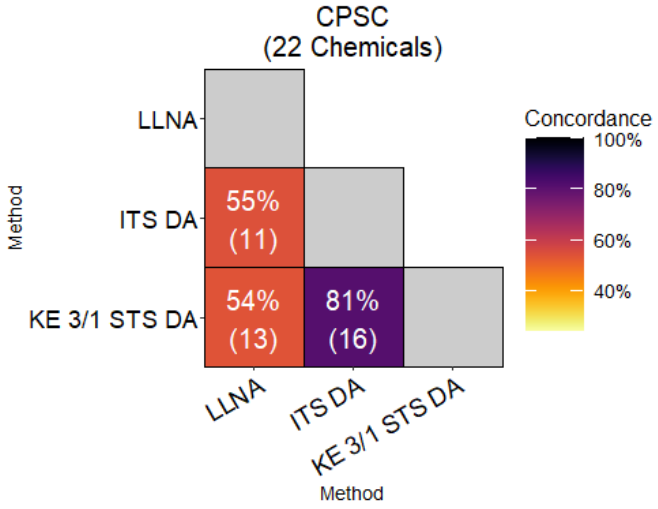


Figure 8. CPSC Concordance of Skin Sensitization Potency Classifications for DAs and LLNA.

3.4. EPA CCTE-Nominated Substances

In all, 12 EPA CCTE-nominated substances were tested for skin sensitization potential using the in vitro methods DPRA, KeratinoSens, and h-CLAT. The hazard and potency classifications for the three assays are summarized in Table 15, along with the OECD Toolbox hazard predictions and the in vivo (animal) hazard predictions. Hazard classifications from the three DAs are also provided, as are potency classifications based on the in vivo animal data and the ITSv2 and KE3/1 DAs. This information is detailed by substance in Table 16.

Two substances did not yield a result with the in vitro methods because of test method limitations, and these are noted in Table 16.

Four substances had concordant results among all three in vitro methods, with two classified as sensitizers and two classified as nonsensitizers (Table 14).

After DPRA, KeratinoSens, and h-CLAT data were evaluated for borderline results for use in the 2o3 DA, 17-36% of the outcomes were borderline or inconclusive (Table 16). There were two (17%) borderline or inconclusive results for DPRA and KeratinoSens and four (36%) borderline or inconclusive results for h-CLAT (Table 15). Inconclusive results occur when the test guideline criteria for positive or negative results are not met or when all three runs of a test method had different results (i.e., positive, negative, borderline) during 2o3 borderline evaluation.

Table 14. EPA CCTE-Nominated Substances Concordant in All In Vitro Assays

Nonsensitizers (by all in vitro methods)

BRTIV Number	CASRN	Chemical Name
BRTIV-114	591-87-7	Allyl acetate
BRTIV-135	4230-97-1	Allyl octanoate

Sensitizers (by all in vitro methods)

BRTIV Number	CASRN	Chemical Name
BRTIV-51	2657-25-2	4'-Hydroxychalcone
BRTIV-54	10373-78-1	Camphorquinone

Nine of the substances were predicted by QSAR Toolbox v4.5 to be sensitizers; all substances were within the QSAR Toolbox applicability domain. There were three substances predicted to be negative; of these, two substances were out-of-domain and are marked with “INC” in Table 16.

All of the 10 substances tested in the in vitro/in chemico assays had in vivo animal data for hazard classification. For one substance, data were from a modified version of the LLNA that pre-dates the LLNA test guideline. The modified LLNA data were used for the in vivo hazard classification but not for in vivo GHS potency classification.

For the 2o3 DA, all substances produced conclusive results prior to the elimination of borderline results for the individual in vitro methods. After the elimination of borderline results, four substances yielded inconclusive results for the 2o3 DA (Table 15).

For the hazard outcomes of the ITSv2 DA prior to the elimination of borderline results, seven of the 12 substances were predicted to be positive, with the remainder predicted to be negative. In the KE3/1 STS hazard outcomes, seven substances were predicted to be positive and four predicted to be negative (Table 15).

Nine substances had adequate in vivo animal data for potency classification. Of these, five substances had potency classifications from ITSv2 and the same five had KE3/1 STS potency classifications (Table 15). Without regard to animal data, 12 substances had ITSv2 potency classifications and 11 substances had KE3/1 STS potency classifications.

Concordances of skin sensitization hazard classifications are summarized in

A.

B.

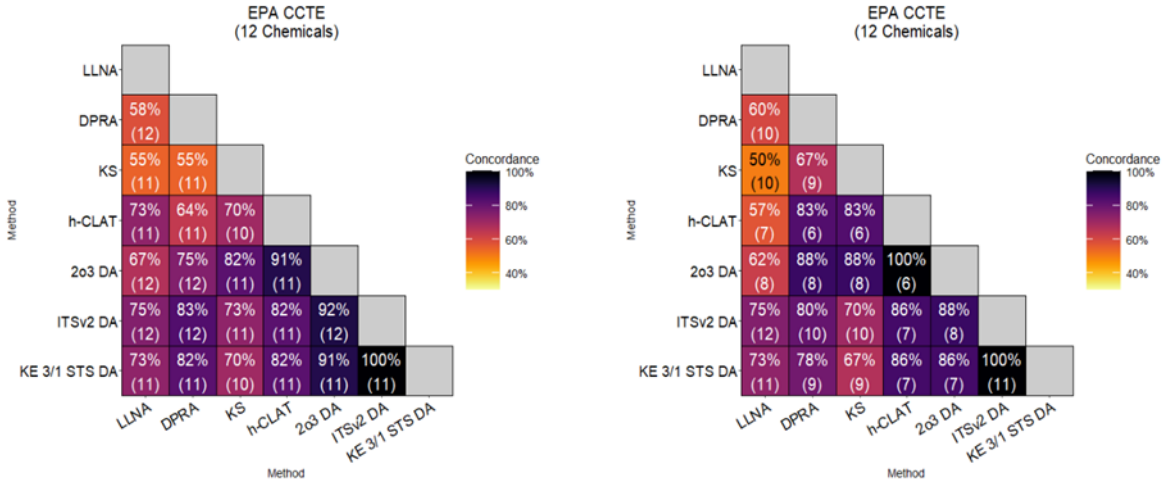


Figure 9 and concordances of potency classifications are summarized in Figure 10. Regardless of whether borderline results were eliminated or not, the same pattern emerged. Concordances were higher among hazard classifications based on in vitro data than between in vitro classifications and those based on in vivo animal (LLNA) data. The highest concordance was among the DAs.

For example,

A.

B.

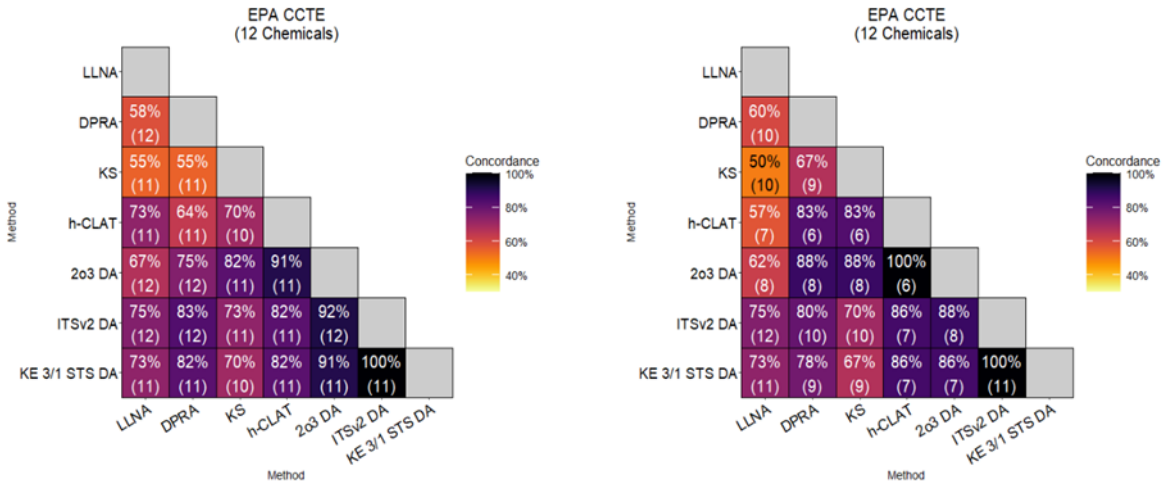


Figure 9A shows that the concordances among h-CLAT, KeratinoSens, and DPRA were 55% to 70% while the concordances among the DAs were 91% to 100%. The concordances of the individual methods with the LLNA ranged from 55% to 73%, and the concordances of the DAs with the LLNA ranged from 67% to 75%. The elimination of borderline results either decreased concordance of the individual test methods with the LLNA or left it unchanged, except for a slight increase in concordance of DPRA with the LLNA data; however, the number of results available for comparison was lower. Regardless of borderline results, the ITSv2 DA had the highest concordance with the LLNA while the KeratinoSens method had the lowest concordance with the LLNA.

For potency, the concordance of the ITSv2 DA with the KE 3/1 STS DA (82%) was higher than the concordance of either DA with the LLNA (44% for both).

Table 15. Summary of EPA CCTE-Nominated Substance Results

Method	Unique Number Evaluated[a]	Initial Number of INC or NA	Final Number of BL, INC, or NA[b]	Number Positive	Number Negative
DPRA Hazard	12	0	2	6	6
KS Hazard	12	1	2	6	4
h-CLAT Hazard	11	0	4	3	4
QSAR TBv4.5 Hazard	12	—	0	9 (9)[c]	3 (1)[c]
In Vivo GHS Hazard	12	—	0	9	3
In Vivo GHS Potency	12	—	3	6 (1A:2, 1B:4)	3
2o3 Hazard	12	0	4	5	3
ITSv2 Hazard	12	—	0	8	4
ITSv2 GHS Potency	12	—	1	7 (1A:2, 1B:5)	4
STS Hazard	12	—	1	7	4
STS GHS Potency	12	—	1	7 (1A:0, 1B:7)	4

Dashes (—) indicate the parameter is not relevant for that test method. Abbreviations: BL = borderline; INC = inconclusive; NA= not applicable or available.

Colors indicate the different types of assays/reference data: Yellow = in chemico/in vitro, Blue = in silico, Green = reference data, Pink = DAs.

[a] Three substances were not tested in at least one of the three in vitro methods. See Table 7 for details.

[b] After the borderline evaluation of the individual test methods (DPRA, KeratinoSens and h-CLAT).

[c] Number in parentheses = number of in-domain predictions.

Table 16. EPA CCTE-Nominated Substance Results

BRTIV Number	DPRA Hazard	KS Hazard	h-CLAT Hazard	QSAR TBv4.5 Hazard	In Vivo Hazard	In Vivo GHS Potency	2o3 Hazard	ITSv2 Hazard	ITSv2 GHS Potency	STS Hazard	STS GHS Potency
BRTIV-47	0	1	BL / 1	INC	1	1B	INC / 1	1	1B	1	1B
BRTIV-49	0	INC / 1	BL / 1	1	1	NA	INC / 1	1	1B	1	1B
BRTIV-51	1	1	1	1	1	1A	1	1	1A	1	1B
BRTIV-54	1	1	1	1	1	1B	1	1	1A	1	1B
BRTIV-58 [a]	1	1	NT	1	1	NA	1	1	INC	NA	NA
BRTIV-82	1	0	0	1	1	1A	0	1	1B	1	1B
BRTIV-98	BL / 0	1	BL / 0	1	0	NC	INC / 0	0	NC	0	NC
BRTIV-114	0	0	0	0	0	NC	0	0	NC	0	NC
BRTIV-127 [b]	BL / 0	INC / BL	0	1	1	1B	INC / 0	0	NC	0	NC
BRTIV-135	0	0	0	1	1	1B	0	0	NC	0	NC
BRTIV-139	1	1	BL / 0	1	0	NC	1	1	1B	1	1B
BRTIV-144	1	0	1	INC	1	NA	1	1	1B	1	1B

Abbreviations: BL = borderline; INC = inconclusive; NA = not applicable or available; NC = not classified; NT= not tested; 1 = positive; 0 = negative.
Colors indicate the different types of assays/reference data: Yellow = in chemico/in vitro, Blue = in silico, Green = reference data, Pink = DAs.
For DPRA, KS, and h-CLAT hazard results, if a BL result differed from the non-BL result, both are listed, separated by a slash. BL is listed first.

[a] Not tested with h-CLAT because it fluoresced at the same wavelength as propidium iodide, which is used for cytotoxicity assessment.

[b] KeratinoSens results were inconclusive because, while there was no positive response observed, test substance solubility was too low to allow testing at concentrations specified in the test guideline.

3.4.1. Concordance of NAMs and Animal Data for EPA CCTE-Nominated Substances

A. **B.**

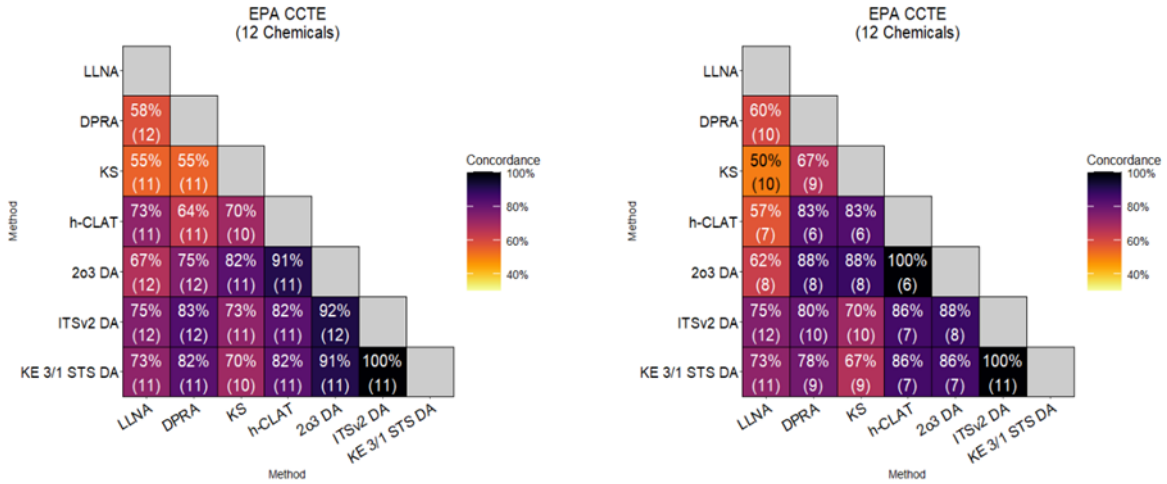


Figure 9. EPA CTTE Concordance of Skin Sensitization Hazard Classifications for NAMs and LLNA.

9A shows concordance of all applicable results while 9B shows the concordance following application of borderline exclusion criteria to individual assays and the 2o3 DA. Inconclusive results are not shown.

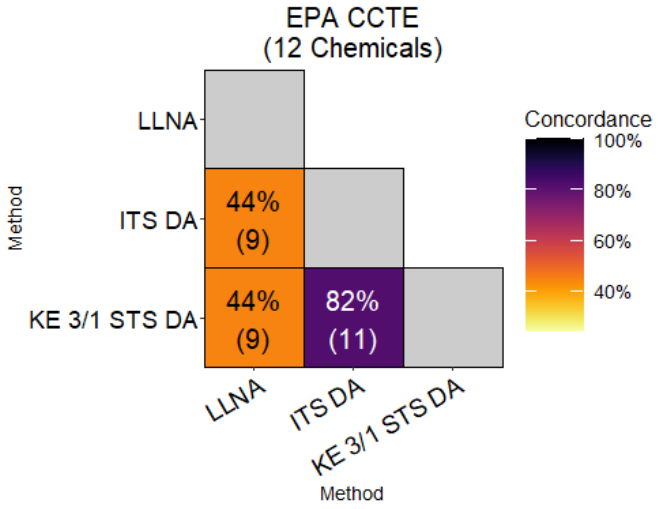


Figure 10. EPA CTTE Concordance of Skin Sensitization Potency Classifications for DAs and LLNA.

3.5. EPA OPP-Nominated Substances

In all, 31 EPA OPP-selected substances were tested for skin sensitization potential using the in vitro methods DPRA, KeratinoSens, and h-CLAT. The hazard and potency classifications for the three assays are summarized in Table 18, along with the OECD Toolbox hazard predictions and the in vivo (animal) hazard predictions. Hazard classifications from the three DAs are also provided, as are potency classifications based on the in vivo animal data and the ITSv2 and KE3/1 DAs. This information is detailed by substance in Table 19.

Ten substances produced inconclusive results from in vitro methods because of test method limitations, and these are noted in Table 19.

Table 17. EPA OPP-Nominated Substances Concordant in All In Vitro Assays

Nonsensitizers (by all in vitro methods)

BRTIV Number	CASRN	Chemical Name
BRTIV-81	1918-00-9	Dicamba
BRTIV-169	NA	RoundUp Precision Gel Weed and Grass Killer (Glyphosphate isopropyl amine)
BRTIV-173	NA	IMA-jet 10 (imidacloprid)
BRTIV-176	NA	Monterey Garden Phos (Phosphorous acid as mon- and di-potassium salts of phosphorous acid)
BRTIV-179	NA	Tepera Fungicide

Sensitizers (by all in vitro methods)

BRTIV Number	CASRN	Chemical Name
BRTIV-119	121-75-5	Malathion
BRTIV-172	NA	Final Soft Bail with Limitrack (Brodifacoum)
BRTIV-181	NA	Quadris Top SBX Fungicide
BRTIV-183	NA	Aquastrike
BRTIV-190	NA	Aquatabs

Within the OPP-nominated substances, five substances were classified as sensitizers by all three methods and five substances were classified as nonsensitizers by all three methods (Table 17).

After DPRA, KeratinoSens, and h-CLAT data were evaluated for borderline results for use in the 2o3 DA, 32–48% of the outcomes were borderline or inconclusive (Table 19). There were 10 (32%) borderline results and one inconclusive result for DPRA, 15 (48%) borderline or inconclusive results for KeratinoSens, and 11 (35%) borderline or inconclusive results for h-CLAT (Table 18).

QSAR Toolbox v4.5 hazard predictions were made for only six of the EPA OPP substances. An additional two substances yielded inconclusive results, and the remainder were assigned an NA because they were mixtures of unknown composition and could not effectively be assessed. There were five positive predictions within the applicability domain (marked with a "1" in Table 19). There were three negative predictions, but only one was in domain; the other two substances were out of the applicability domain (marked with "0" or "INC" in Table 19, respectively).

All 31 substances had in vivo animal data for hazard classification; five had insufficient data for potency classification.

For the 2o3 DA, even before the evaluation of borderline results, four substances had inconclusive outcomes because they were inconclusive in at least one of the three in vitro methods; these are noted in Table 19. After the borderline evaluation, 13 additional substances

had inconclusive results due to borderline or inconclusive results for DPRA, KeratinoSens, or h-CLAT.

For the hazard outcomes of the ITSv2 DA, four substances had inconclusive calls and one substance (Diffense®, BRTIV-187) had an NA result. Diffense also had an NA hazard outcome for the KE3/1 STS DA because it was inconclusive in at least one of the three in vitro methods. These are all noted in Table 19.

Concordance of skin sensitization hazard classifications are summarized in

A. **B.**

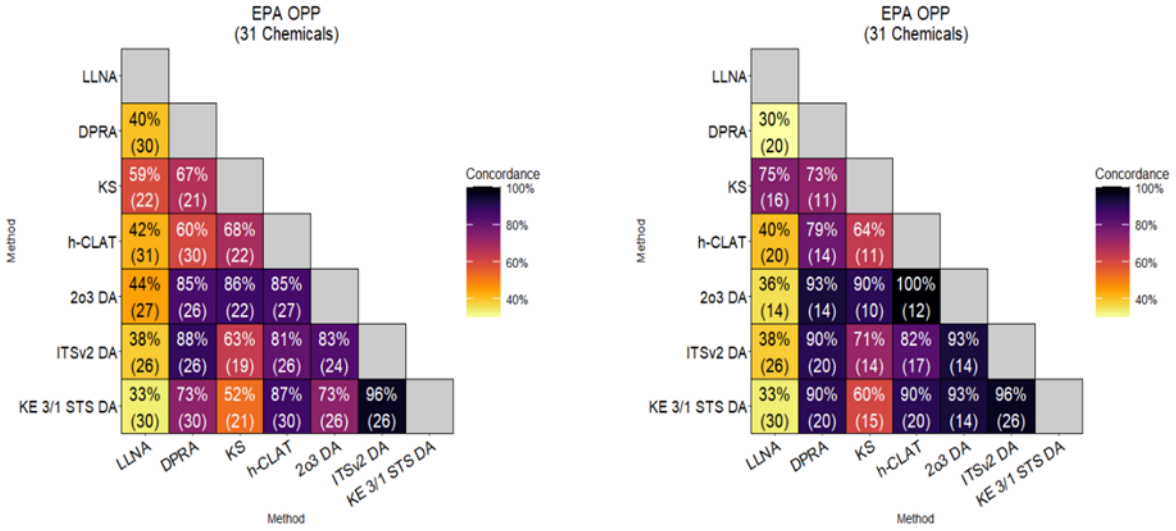


Figure 11. Regardless of whether borderline results were eliminated or not, a similar pattern emerged: the highest concordance of hazard classifications was among the DAs. For example,

A. **B.**

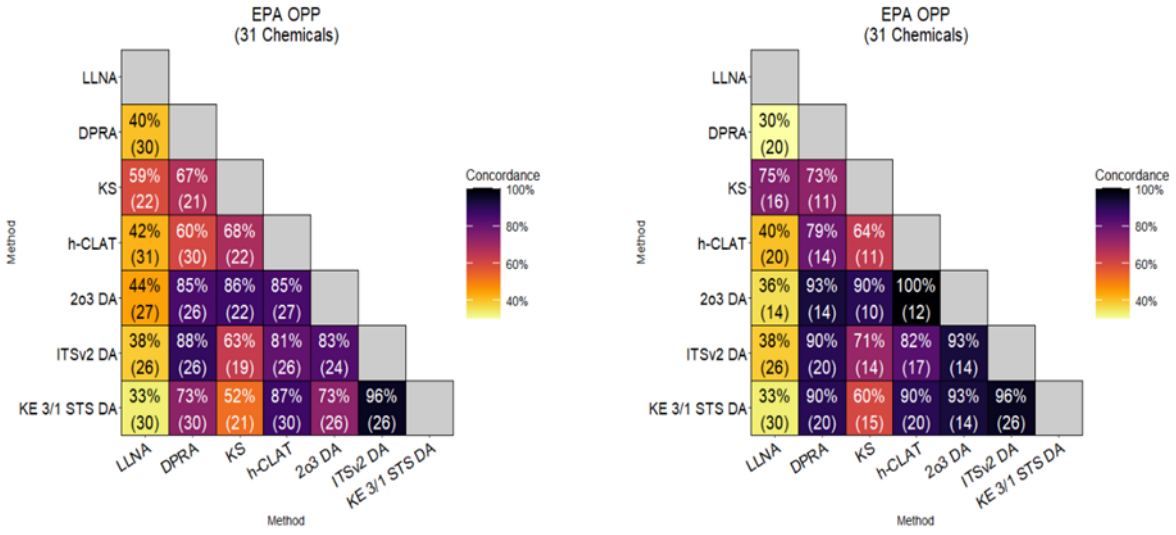


Figure 11A shows that the concordances of classifications based on data from h-CLAT, KeratinoSens, and DPRA were 60% to 68% while the concordance among the DAs was 73% to 96%.

A. **B.**

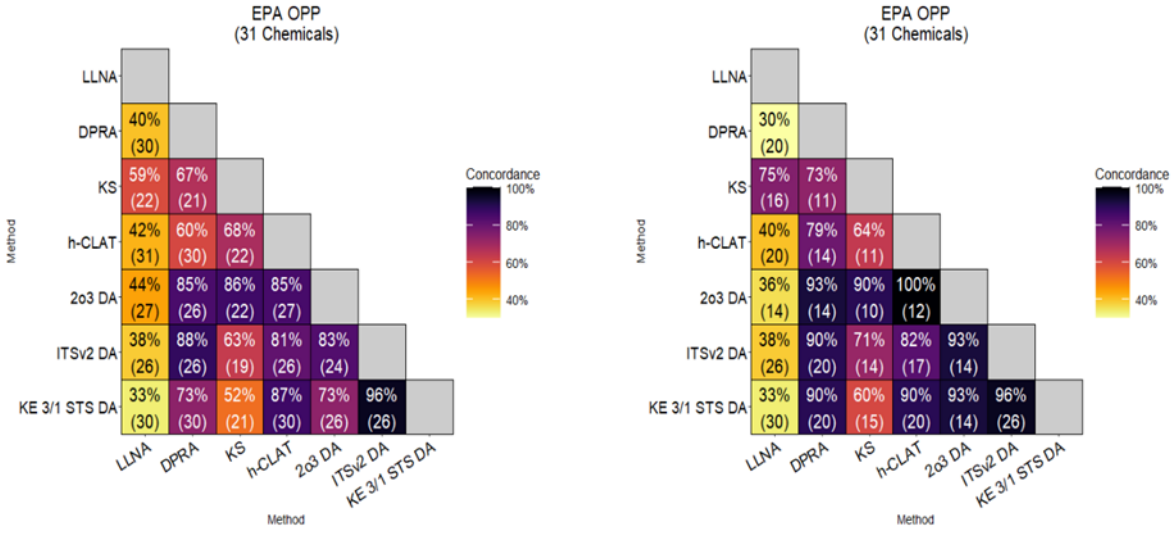


Figure 11A also shows that the concordances of classifications based on data from the individual in vitro methods with the LLNA (40% to 59%) were typically higher than the concordance of the DA hazard classifications with in vivo LLNA classifications (33% to 44%). KeratinoSens classifications had the highest concordance with those based on the LLNA at 59%. This pattern remained after borderline results were removed (

A. **B.**

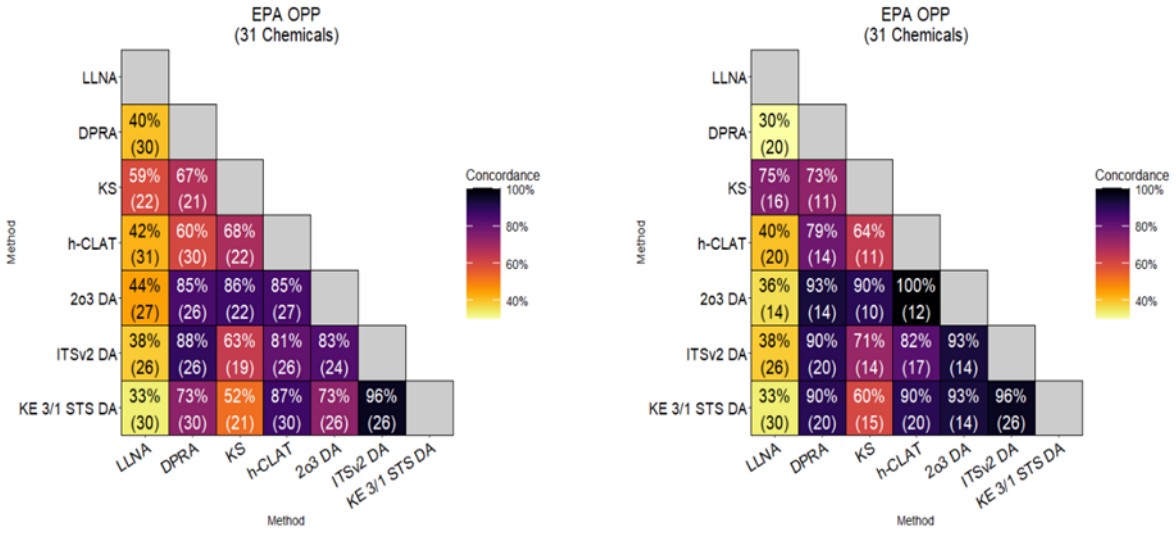


Figure 11B). The elimination of borderline results increased the concordance among the individual test methods and the concordance among the DAs. The concordance of the individual in vitro methods with the LLNA, increased for some, decreased for others, and stayed the same for some.

Propanil was the only substance of the six sensitizers that had adequate in vivo animal data for potency classification; thus, the remaining five sensitizers are shown with NA results in Table 19. Without comparison to animal data, 25 substances had conclusive ITSv2 potency classifications and five substances had inconclusive results. Diffense did not produce a result (NA) because it was inconclusive in at least one of the three in vitro methods. Of the 25 substances with conclusive results, one was classified as a 1A sensitizer, 17 were classified as 1B sensitizers, and seven were Not Classified (negative) (Table 17). Thirty substances had KE3/1 STS potency classifications, 24 were classified as 1B sensitizers, and six were Not Classified (negative) (Table 17).

The concordances of potency classifications are summarized in Figure 12. The concordance of the ITSv2 DA with the KE 3/1 STS DA was much higher (92%) than the concordance of either DA with the LLNA (24-32%).

Table 18. Summary of EPA OPP-Nominated Substance Results

Method	Unique Number Evaluated	Initial Number of INC or NA	Final Number of BL, INC, or NA[a]	Number Positive	Number Negative
DPRA Hazard	31	1	11	15	5
KS Hazard	31	9	15	5	11
h-CLAT Hazard	31	0	11	14	6
QSAR TBv4.5 Hazard	31	—	23	5 (5)[b]	3 (1)[b]
In Vivo GHS Hazard	31	—	0	6	25
In Vivo GHS Potency	31	—	5	1 (1A:0, 1B:1)	25
2o3 Hazard	31	4	17	10	4
ITSv2 Hazard	31	—	5	19	7
ITSv2 GHS Potency	31	—	6	18 (1A:1, 1B:17)	7
STS Hazard	31	—	1	24	6
STS GHS Potency	31	—	1	24 (1A:0, 1B:24)	6

Dashes (—) indicate the parameter is not relevant for that test method. Abbreviations: BL = borderline; INC = inconclusive; NA = not applicable or available.

Colors indicate the different types of assays/reference data: Yellow = in chemico/in vitro, Blue = in silico, Green = reference data, Pink = DAs.

[a] After the borderline evaluation of the individual test methods (DPRA, KeratinoSens and h-CLAT).

[b] Number in parentheses = number of in-domain predictions.

Table 19. EPA OPP-Nominated Substance Results

BRTIV Number	DPRA Hazard	KS Hazard	h-CLAT Hazard	QSAR TBv4.5 Hazard	In Vivo Hazard	In Vivo GHS Potency	2o3 Hazard	ITSv2 Hazard	ITSv2 GHS Potency	STS Hazard	STS GHS Potency
BRTIV-75	BL / 0	BL / 1	0	1	0	NC	0	0	NC	0	NC
BRTIV-76[a, b]	BL / 0	INC	INC / 1	1	1	1B	INC	1	1B	1	1B
BRTIV-78	BL / 0	0	1	NA	0	NC	INC / 0	INC	INC	1	1B
BRTIV-79[a]	1	INC	1	1	0	NC	1	1	1A	1	1B
BRTIV-80[a,b]	1	INC	BL / 0	INC	0	NC	INC	1	1B	1	1B
BRTIV-81	0	0	0	INC	0	NC	0	0	NC	0	NC
BRTIV-110	BL / 0	0	1	0	0	NC	INC / 0	0	NC	1	1B
BRTIV-119	1	1	BL / 1	1	0	NC	1	1	1B	1	1B
BRTIV-133	0	BL / 1	BL / 1	1	0	NC	1	1	1B	1	1B
BRTIV-169	0	0	0	NA	0	NC	0	0	NC	0	NC
BRTIV-170	BL / 0	BL / 0	1	NA	0	NC	0	INC	INC	1	1B
BRTIV-171[a]	1	INC	INC / 1	NA	0	NC	1	1	1B	1	1B
BRTIV-173	BL / 0	BL / 0	BL / 0	NA	1	NA	0	0	NC	0	NC
BRTIV-174[a]	1	INC	1	NA	0	NC	1	1	1B	1	1B
BRTIV-175[a b]	BL / 0	INC	BL / 1	NA	0	NC	INC	INC	INC	1	1B
BRTIV-176	0	0	0	NA	0	NC	0	0	NC	0	NC
BRTIV-177[a]	1	INC	1	NA	0	NC	1	1	1B	1	1B
BRTIV-172	1	1	1	NA	0	NC	1	1	1B	1	1B
BRTIV-178	1	0	0	NA	0	NC	0	1	1B	1	1B
BRTIV-179	BL / 0	0	BL / 0	NA	0	NC	0	0	NC	0	NC
BRTIV-180	BL / 1	0	1	NA	0	NC	INC / 1	1	1B	1	1B
BRTIV-181	1	1	1	NA	0	NC	1	1	1B	1	1B
BRTIV-182	1	0	1	NA	0	NC	1	1	1B	1	1B
BRTIV-183	1	1	1	NA	1	NA	1	1	1	1	1B
BRTIV-184	1	0 INC	0	NA	1	NA	INC / 0	1	1B	1	1B
BRTIV-185	0	INC / 0	1	NA	1	NA	INC / 0	1	1B	1	1B
BRTIV-186[a]	1	INC / BL	1	NA	0	NC	1	1	1B	1	1B
BRTIV-187[b,c]	INC	0	INC / 0	NA	0	NC	0	NA	NA	NA	NA
BRTIV-188	1	0	BL / 0	NA	0	NC	INC / 0	1	1B	1	1B
BRTIV-189[a,b]	BL / 0	INC	1	NA	1	NA	INC	INC	INC	1	1B
BRTIV-190	1	1	BL / 1	NA	0	NC	1	1	1B	1	1B

Abbreviations: BL = borderline; INC = inconclusive; NA = not applicable or available; NC = not classified; 1 = positive; 0 = negative.

Colors indicate the different types of assays/reference data: Yellow = in chemico/in vitro, Blue = in silico, Green = reference data, Pink = DAs.

For DPRA, KS, and h-CLAT hazard results, if a BL result differed from the non-BL result, both are listed, separated by a slash. BL is listed first.

[a] KeratinoSens results were inconclusive because, while there was no positive response observed, test substance solubility was too low to allow testing at concentrations specified in the test guideline.

[b]Inconclusive or NA hazard or potency classification because the test substance was either not tested in or was inconclusive in at least one of the three in vitro methods. [c]Inconclusive DPRA results because substance co-eluted with the cysteine peptide.

3.5.1. Concordance of NAMs and Animal Data for EPA OPP-Nominated Substances

A. **B.**

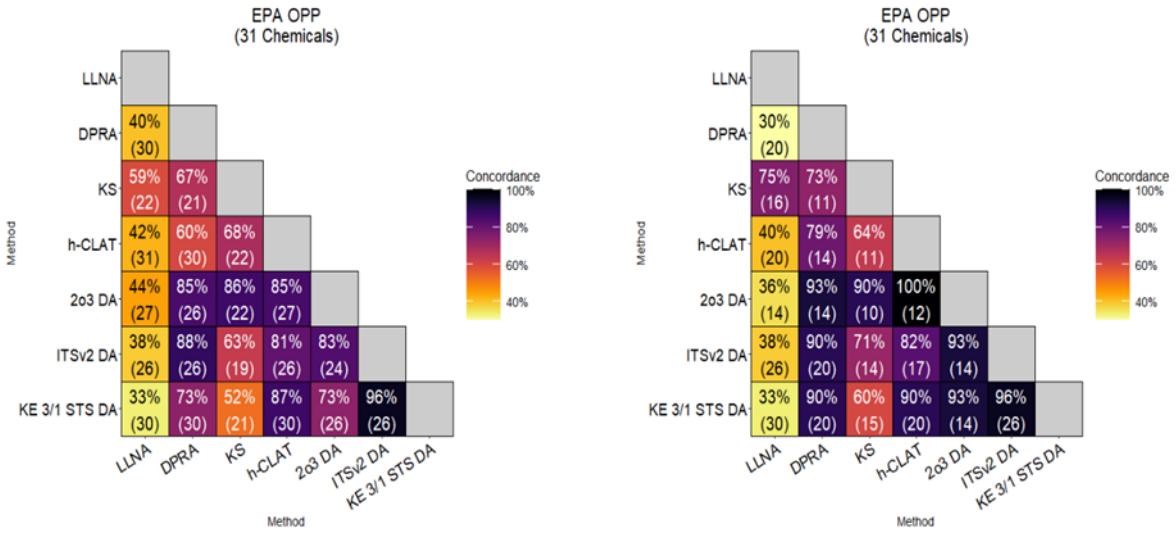


Figure 11. EPA OPP Concordance of Skin Sensitization Hazard Classifications for NAMs and LLNA.

11A shows concordance of results without the evaluation of borderline results while 11B shows the concordance after borderline results are eliminated. Inconclusive results are not shown.

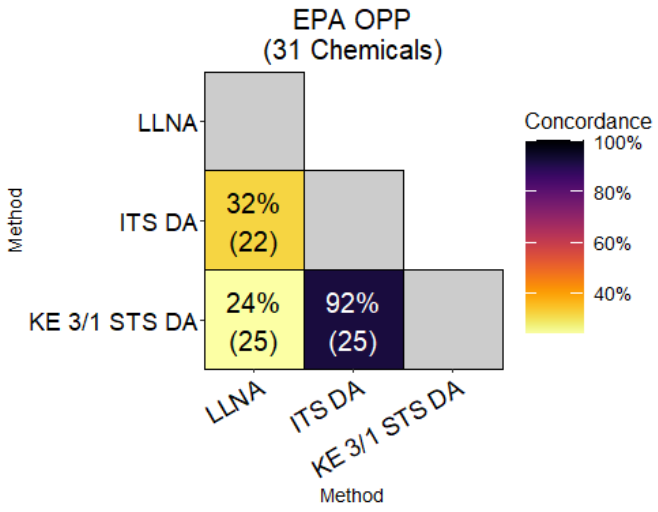


Figure 12. EPA OPP Concordance of Skin Sensitization Potency Classifications for DAs and LLNA.

3.6. EPA OPPT-Nominated Substances

In all, 50 EPA OPPT selected substances were tested for skin sensitization potential using the in vitro methods DPRA, KeratinoSens, and h-CLAT. The hazard and potency classifications for the three assays are summarized in Table 20, along with the OECD Toolbox hazard predictions and the in vivo (animal) hazard predictions. Hazard classifications from the three DAs are also provided, as are potency classifications based on the in vivo animal data and the ITSv2 and KE3/1 DAs. This information is detailed by substance in Table 22.

Seven substances yielded inconclusive results for DPRA or KeratinoSens because of test method limitations, which are noted in Table 22. All substances tested in h-CLAT yielded conclusive results.

Within the OPPT-nominated substances, three were classified as nonsensitizers by all three methods and 11 substances were classified as sensitizers by all three methods (Table 21).

After DPRA, KeratinoSens, and h-CLAT data were evaluated for borderline results, 19-34% of the outcomes were borderline or inconclusive (Table 22). There were 10 (21%) borderline or inconclusive results for DPRA, 9 (19%) borderline or inconclusive results for KeratinoSens, and 16 (34%) borderline or inconclusive results for h-CLAT.

OECD QSAR Toolbox v4.5 skin sensitization hazard predictions were not obtained for seven of the EPA OPPT substances. These are marked "NA" in Table 22, with details provided in the table footnotes. There were 25 positive predictions, with two of these outside the applicability domain. There were 18 negative predictions, 10 of which were out-of-domain. Predictions that were outside the applicability domain are marked with "INC" in Table 22.

All 50 substances had in vivo animal data for hazard classification. LLNA data for 10 substances were from modified versions of the LLNA that that did not use radioactive markers. The modified LLNA data were used for the in vivo hazard classification but not for in vivo GHS potency classification. Eight other substances had insufficient information to determine GHS potency classification. Thus, 32 substances had sufficient LLNA data for potency classification.

For the 2o3 DA, even before the evaluation of borderline results, five substances had inconclusive ("INC") or NA hazard classification outcomes because the test substance was either not tested in or was inconclusive in at least one of the three in vitro methods; these are noted in Table 22. The 2o3 DA classified 30 substances as sensitizers and 15 substances as nonsensitizers. After the evaluation of borderlines, 18 substances had inconclusive or NA results, 22 substances were classified as sensitizers and 10 substances were classified as nonsensitizers (Table 22).

For the hazard outcomes of the ITSv2 DA, nine substances had inconclusive or NA calls due to missing or inconclusive results from one or more methods. Three of these substances had an NA hazard outcome because the result from only one of the three methods was available. The ITSv2 classified 30 substances as sensitizers and 11 substances as nonsensitizers. Three substances had an NA hazard outcome for the KE3/1 STS because they were not tested in h-CLAT. The KE 3/1 STS classified 38 substances as sensitizers and nine substances as nonsensitizers. See Table 21 for a summary of sensitization results.

For potency classifications, 40 substances had ITSv2 potency classifications and 47 substances had KE3/1 STS potency classifications. The in vivo data classified five substances as

1A sensitizers, 21 substances as 1B sensitizers and six substances as Not Classified (negative). The ITSv2 DA classified seven substances as 1A sensitizers, 22 substances as 1B sensitizers and 11 substances as Not Classified (negative). The KE 3/2 STS DA classified six substances as 1A sensitizers, 32 substances as 1B sensitizers and nine substances as Not Classified (negative). See Table 20 for a summary of potency classification results.

Concordances of skin sensitization hazard classifications are summarized in

A. **B.**

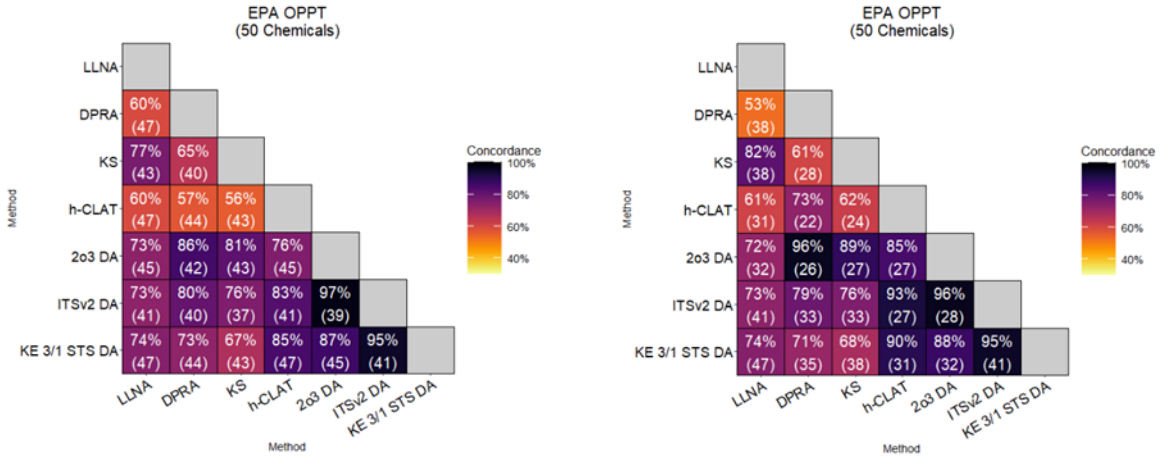


Figure 13. Regardless of whether borderline results were eliminated, the same pattern emerged. Concordances were higher among hazard classifications based on data from the three in vitro tests than between those based on any of the in vitro tests and classifications based on in vivo animal (LLNA) data. The highest concordance was among the DAs.

A. **B.**

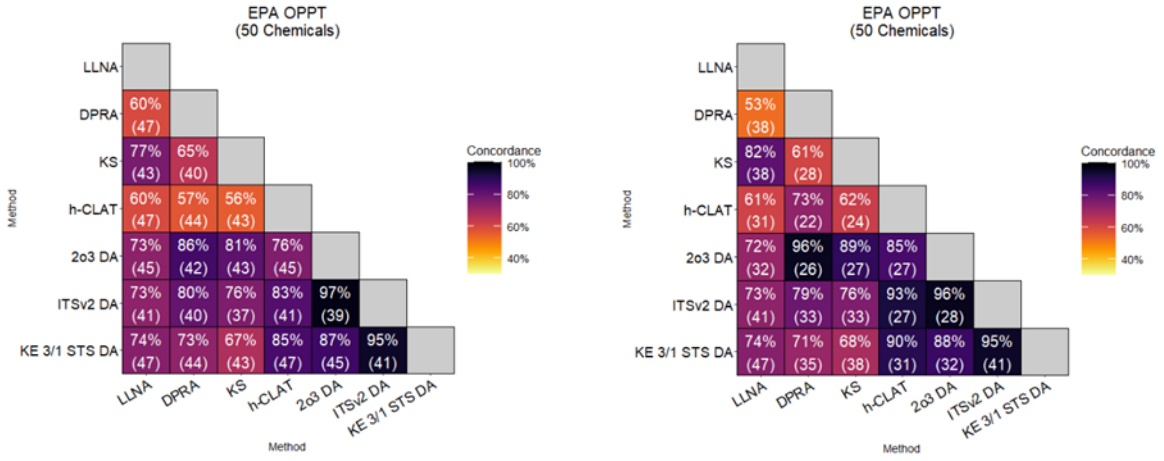


Figure 13A shows that the concordances among h-CLAT, KeratinoSens, and DPRA were 56% to 65% while the concordances among the DAs were 87% to 97%. The concordances with the

LLNA ranged from 60% to 77% for the individual methods and 73% to 74% for the DAs. When borderline results were eliminated, the concordance with LLNA typically remained approximately the same (

A.

B.

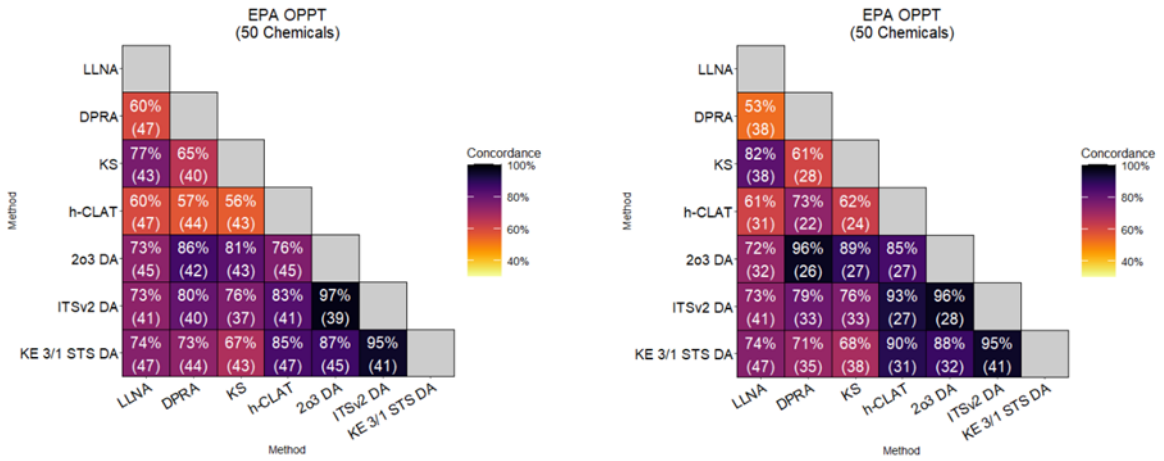


Figure 13B), except for a decrease in concordance of DPRA with the LLNA data and an increase in the concordance of KeratinoSens with the LLNA; however, the number of results available for comparison was lower. Regardless of borderline results, KeratinoSens had the highest concordance with the LLNA while the DPRA (and h-CLAT, prior to removal of borderline results) had the lowest concordance with the LLNA.

The concordances of potency classifications are summarized in Figure 14. The concordance of the ITSv2 DA with the KE 3/1 STS DA (70%) was much higher than the concordance of either DA with the LLNA (33% -57%). The KE 3/1 STS DA had the highest concordance with the LLNA potency classifications.

Table 20. Summary of EPA OPPT-Nominated Substance Results

Method	Unique Number Evaluated [a]	Initial Number of INC or NA	Final Number of BL, INC, or NA [b]	Number Positive	Number Negative
DPRA Hazard	48	1	10	18	20
KS Hazard	47	4	9	30	8
h-CLAT Hazard	47	0	16	21	10
QSAR TBv4.5 Hazard	50	—	7	25 (23)	18 (8)
In Vivo GHS Hazard	50	—	0	43	7
In Vivo GHS Potency	50	—	18	26 (1A:5, 1B:21)	6
2o3 Hazard	50	5	18	22	10
ITSv2 Hazard	50	—	9	30	11
ITSv2 GHS Potency	50	—	10	29 (1A:7, 1B:22)	11
STS Hazard	50	—	3	38	9
STS GHS Potency	50	—	3	38 (1A:6, 1B:32)	9

Dashes (—) indicate the parameter is not relevant for that test method. Abbreviations: BL = borderline; INC = inconclusive; NA = not applicable or available.

Colors indicate the different types of assays/reference data: Yellow = in chemico/in vitro, Blue = in silico, Green = reference data, Pink = DAs.
[a] Not tested (2).
[b] After the borderline evaluation of the individual test methods (DPRA, KeratinoSens and h-CLAT). [c] Number in parentheses = number of in-domain predictions.

Table 21. EPA OPPT-Nominated Substances Concordant in All In Vitro Assays

Nonsensitizers (by all in vitro methods) (3)

BRTIV Number	CASRN	Chemical Name
BRTIV-84	25354-97-6	2-Hexyldecanoic acid
BRTIV-106	111-01-3	Squalane
BRTIV-150	50849-47-3	2-Hydroxy-5-nonylbenzaldoxime

Sensitizers (by all in vitro methods) (11)

BRTIV Number	CASRN	Chemical Name
BRTIV-63	142-31-4	Sodium octyl sulfate
BRTIV-70	14024-63-6	Bis(pentane-2,4-dionato)zinc
BRTIV-104	28961-43-5	Poly(oxy-1,2-ethanediyl),.alpha.-hydro-.omega.-[(1-oxo-2-propenyl)oxy]-, ether with 2-ethyl-2-(hydroxymethyl)-1,3-propanediol (3:1)
BRTIV-108	78-51-3	Trisbutoxyethyl phosphate
BRTIV-112	3031-66-1	3-Hexyne-2,5-diol
BRTIV-113	5208-93-5	3-Methyl-1-(2,6,6-trimethylcyclohex-1-en-1yl)penta-1,4-dien-3-ol
BRTIV-137	66415-55-2	Aminopropyl vinyl ether
BRTIV-138	72676-55-2	1,3,4-Thiadiazole-2(3H)-thione, 5,5'-dithiobis-
BRTIV-147	84100-23-2	2-Propenoic acid, 4-(1,1-dimethylethyl)cyclohexyl ester
BRTIV-149	10436-39-2	1,1,2,3-Tetrachloropropene
BRTIV-160	64265-57-2	1-Aziridinepropanoic acid, 2-methyl-, 2-ethyl-2-((3-(2-methyl-1-aziridinyl)-1-oxopropoxy)methyl)-1,3-propanediyl

Table 22. EPA OPPT-Nominated Substance Results

BRTIV Number	DPRA Hazard	KS Hazard	h-CLAT Hazard	QSAR TBv4.5 Hazard	In Vivo Hazard	In Vivo GHS Potency	2o3 Hazard	ITSv2 Hazard	ITSv2 GHS Potency	STS Hazard	STS GHS Potency
BRTIV-48[a]	0	1	0	1	1	1B	0	0	NC	0	NC
BRTIV-53[b]	BL / 0	0	1	NA	0	NA	INC / 0	INC	INC	1	1B
BRTIV-60	1	1	BL / 1	0	1	NA	INC / 1	1	1B	1	1A
BRTIV-61	NT	1	1	INC	1	NA	1	NA	NA	1	1A
BRTIV-62[c]	BL / 0	1	1	NA	1	1A	1	1	1B	1	1A
BRTIV-63	1	1	1	0	0	NC	1	1	1B	1	1B
BRTIV-66	0	0	BL / 1	INC	1	1B	0	INC	INC	1	1B
BRTIV-67	0	NT	NT	INC	1	1B	NA	NA	NA	NA	NA
BRTIV-70	1	1	1	1	1	1B	1	1	1B	1	1B
BRTIV-72	1	0	INC / 1	1	1	1B	INC / 1	1	1A	1	1B
BRTIV-73[d]	1	INC	1	1	1	1B	1	1	1A	1	1B
BRTIV-74	0	NT	NT	0	1	1B	NA	INC	INC	NA	NA
BRTIV-84	BL / 0	0	0	0	1	1B	0	0	NC	0	NC
BRTIV-91	0	1	BL / 1	1	1	1B	INC / 1	1	1B	1	1B
BRTIV-94	1	INC / 0	1	1	1	1B	1	1	1A	1	1B
BRTIV-99	0	1	BL / 0	1	1	1B	INC / 0	0	NC	0	NC
BRTIV-102	0	1	BL / 0	1	1	1A	INC / 0	0	NC	0	NC
BRTIV-104	1	1	INC / 1	1	1	NA	1	1	1A	1	1B
BRTIV-106	0	0	0	0	0	NC	0	0	NC	0	NC
BRTIV-107[d,e]	BL / 1	INC	0	0	1	1B	INC	0	NC	1	1B
BRTIV-108	BL / 1	1	INC / 1	1	1	1B	1	1	1B	1	1B
BRTIV-112	1	1	1	1	1	NA	1	1	1B	1	1B
BRTIV-113	1	1	1	1	1	1A	1	1	1B	1	1B
BRTIV-116[f]	INC	1	1	0	1	NA	1	INC	INC	1	1B
BRTIV-118	1	1	0	1	1	NA	1	1	1B	1	1B
BRTIV-122	0	NT	NT	INC	1	NA	NA	NA	NA	NA	NA
BRTIV-123	0	1	INC / 1	INC	1	1B	INC / 1	1	1B	1	1B
BRTIV-124	0	0	1	INC	0	NC	0	INC	INC	1	1B
BRTIV-125[b]	BL / 0	1	0	NA	0	NC	INC / 0	0	NC	0	NC

Table 22 (Continued). EPA OPPT-Nominated Substances Results

BRTIV Number	DPRA Hazard	KS Hazard	h-CLAT Hazard	QSAR TBv4.5 Hazard	In Vivo Hazard	In Vivo GHS Potency	2o3 Hazard	ITSv2 Hazard	ITSv2 GHS Potency	STS Hazard	STS GHS Potency
BRTIV-126	0	1	0	INC	1	1B	0	0	NC	0	NC
BRTIV-128	1	1	BL / 0	INC	1	1B	1	1	1B	1	1B
BRTIV-129	1	1	0	1	1	1B	1	1	1B	1	1B
BRTIV-130	0	1	BL / 1	INC	1	NA	INC / 1	1	1B	1	1A
BRTIV-131	0	0	INC / 1	0	1	1B	0	0	NC	1	1B
BRTIV-137	BL / 1	1	1	INC	1	NA	1	1	1B	1	1B
BRTIV-138	1	1	1	1	1	NA	1	1	1A	1	1B
BRTIV-143[g]	0	INC / 1	1	NA	0	NC	INC / 1	1	1B	1	1B
BRTIV-147	1	1	1	1	1	NA	1	1	1B	1	1B
BRTIV-148	0	1	0	INC	1	1B	0	0	NC	0	NC
BRTIV-149	1	1	1	1	1	1B	1	1	1A	1	1B
BRTIV-150	0	BL / 0	0	1	1	NA	0	0	NC	0	NC
BRTIV-151[c]	1	1	INC / 0	NA	1	1A	1	1	1B	1	1B
BRTIV-152	BL / 1	1	INC / 0	1	1	1A	1	1	1B	1	1B
BRTIV-153	NT	1	1	1	1	NA	1	1	INC	1	1B
BRTIV-154[d,b]	1	INC	1	NA	1	1B	1	1	1B	1	1B
BRTIV-155	0	0	1	1	1	NA	0	1	1B	1	1B
BRTIV-157[a,g]	0	1	1	NA	1	NA	1	1	1B	1	1A
BRTIV-158[d,e]	1	INC	INC / 0	1	1	NA	INC	1	1B	1	1B
BRTIV-159	BL / 0	INC / 0	INC / 1	INC	0	NC	0	INC	INC	1	1B
BRTIV-160	1	INC / 1	1	1	1	NA	1	1	1A	1	1A

Abbreviations: BL = borderline; INC = inconclusive; NA = not applicable or available; NC= not classified; NT= not tested; 1 = positive; 0 = negative.
Colors indicate the different types of assays/reference data: Yellow = in chemico/in vitro, Blue = in silico, Green = reference data, Pink = DAs.
For DPRA, KS, and h-CLAT hazard results, if a BL result differed from the non-BL result, both are listed, separated by a slash. BL is listed first.

[a] DPRA results were inconclusive because the test substance at less than 100 mM due to solubility limitations and had minimal reactivity.

[b] QSAR hazard could not be predicted because substance was an inorganic compound.

[c] QSAR hazard could not be predicted because there was an insufficient number of similar chemicals with skin sensitization data.

[d] KeratinoSens results were inconclusive because, while there was no positive response observed, test substance solubility was too low to allow testing at concentrations specified in the test guideline.

[e] Hazard or potency classification was inconclusive or NA because the test substance was either not tested in or was inconclusive in at least one of the three in vitro methods.

[f] DPRA results were inconclusive because the test substance co-eluted with the cysteine peptide.

[g] QSAR hazard could not be predicted because substance did not have a definitive chemical structure.

3.6.1. Concordance of NAMs and Animal Data for EPA OPPT-Nominated Substances

A. **B.**

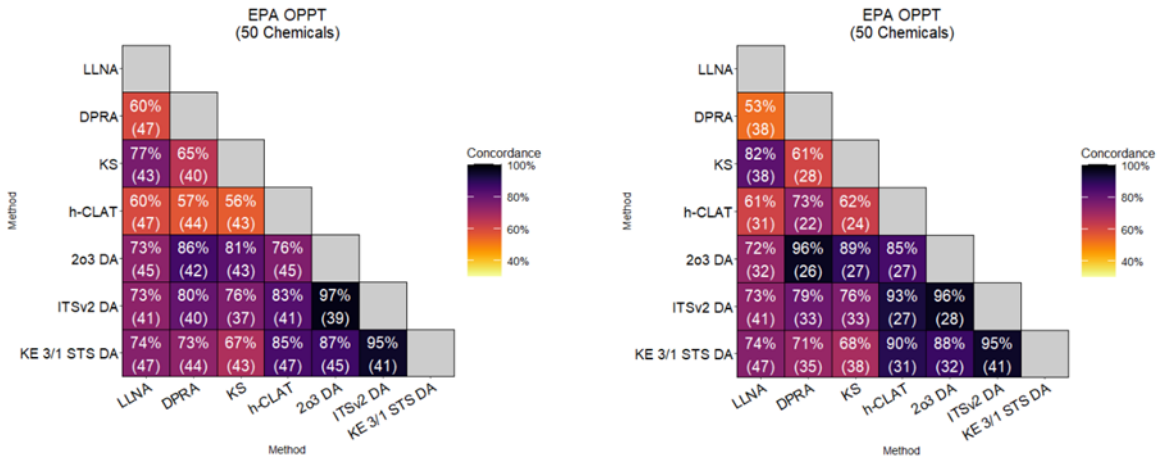


Figure 13. EPA OPPT Concordance of Skin Sensitization Hazard Classifications for NAMs and LLNA.

13A shows concordance of all applicable results while 13B shows the concordance following application of borderline exclusion criteria to individual assays and the 2o3 DA. Inconclusive results are not shown.

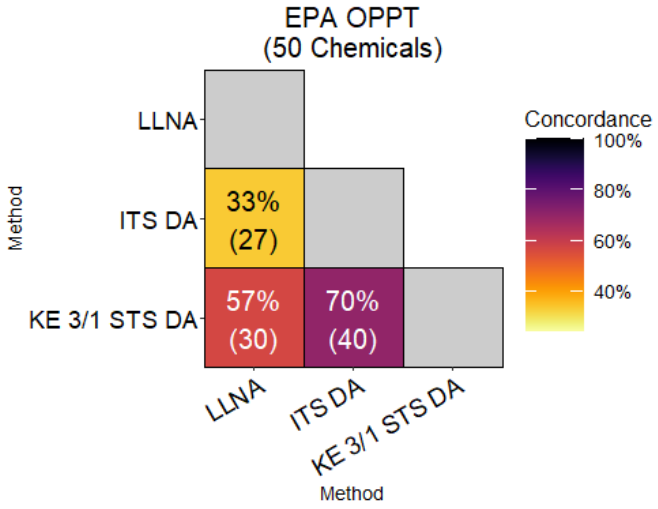


Figure 14. EPA OPPT Concordance of Skin Sensitization Potency Classifications for DAs and LLNA.

3.7. Concordance of NAMs and Human Data

Of the 181 substances tested, human reference data were available for 24 substances (Table 23). Table 24 shows the skin sensitization hazard classifications for the human data along with those from DPRA, KeratinoSens, h-CLAT, OECD Toolbox, LLNA, 2o3 DA, ITSv2 DA, and KE 3/1 STS DA. DPRA, KeratinoSens, and h-CLAT results are reported both with and without the evaluation of borderline results. Four substances had borderline DPRA results, three substances had borderline or inconclusive h-CLAT results, and one substance had inconclusive KeratinoSens results. The three substances with inconclusive results for the 2o3 DA had borderline DPRA results with discordant KeratinoSens and h-CLAT results. There were inconclusive results for two substances (from KeratinoSens and h-CLAT), but these did not affect the 2o3 outcomes because there were still two concordant tests available to make the 2o3 decision. DTT had the highest number of nominated chemicals, with 12, while one to five chemicals were nominated by other agency partners. The following paragraphs and outcome evaluations are derived from the total list of 181 tested substances.

Data for three substances were from tests on workers, patients, or previously sensitized subjects rather than from predictive patch test data, while data for the substance nominated by FDA came from a drug information package for METVIXIA; these are noted in Table 24. Twelve substances were classified as sensitizers and 12 were classified as nonsensitizers. While data for 19 substances were useful for potency classification, data for five sensitizers were useful for hazard classification only. Five substances were classified as 1A sensitizers, two substances were classified as 1B sensitizers, and 12 substances were classified as Not Classified (negative). Data and source information are provided in Appendix A.

After DPRA and h-CLAT data were evaluated for borderline results, 13-17% of the outcomes were borderline or inconclusive (Table 24). There were no borderline results for KeratinoSens, however, there was one inconclusive result because of test method limitations.

OECD QSAR Toobox v4.5 hazard predictions were not made for four of the human reference data substances because of test method limitations. These are marked “NA” in Table 24, with details included in the footnotes. There were 14 positive predictions with none outside the applicability domain. There were six negative predictions, of which two were out-of-domain. Predictions that were outside the applicability domain are marked with “INC” in Table 24.

Twenty-two substances had in vivo animal data for hazard classification. Data for two substances were from modified versions of the LLNA that measure lymphocyte proliferation ex vivo. The modified LLNAs were used for hazard classification but not for GHS potency classification. Seventeen substances were LLNA sensitizers, and five substances were nonsensitizers. The in vivo data classified six substances as 1A sensitizers, eight substances as 1B sensitizers and five substances as Not Classified (negative).

For the 2o3 DA, three substances had inconclusive outcomes after the evaluation of borderline results: Iso E Super, zinc diethyldithiocarbamate, and ethyl formate. All three had borderline DPRA results with discordant KeratinoSens and h-CLAT hazard classifications. The 2o3 DA yielded no inconclusive results before borderline evaluation. Nineteen substances were sensitizers and five substances were nonsensitizers. The borderline evaluation reduced the number of sensitizers by one and the number of nonsensitizers by two. Hazard predictions from both the ITSv2 DA or KE3/1 STS produced no inconclusive results, either before or after

borderline evaluation. The ITSv2 DA classified 20 substances as sensitizers and four substances as nonsensitizers, while the KE 3/1 STS classified 21 substances as sensitizers and three substances as nonsensitizers.

Nineteen substances had adequate in vivo animal data for potency classification. All 24 substances had potency classifications for ITSv2 and 18 had KE3/1 STS potency classifications. The ITSv2 DA classified six substances as 1A sensitizers, 13 substances as 1B sensitizers, and four substances as Not Classified (negative); one substance was inconclusive. The KE 3/2 STS DA classified eight substances as 1A sensitizers, 13 substances as 1B sensitizers and three substances as Not Classified (negative).

Concordances of skin sensitization hazard classifications are summarized in **Error! Reference source not found.**. Regardless of whether borderline results were eliminated, the same pattern emerged. Concordance was higher among classifications based on NAMs than between classifications based on most NAMs and classifications based on human reference data. The highest concordances were among the DAs. **Error! Reference source not found.**A shows that the concordance among h-CLAT, KeratinoSens, and DPRA was 75% to 83% while the concordance among the DAs was 92% to 96%. The concordance with the LLNA ranged from 73% to 82% for the individual methods and 82% to 86% for the DAs. While the concordance of human and LLNA data was 64%, the concordance of the individual methods with human data was 58% to 78% and the concordance of the DAs with human data was 62% to 71%. Thus, with the exception of h-CLAT and the KE31/STS DA, all NAMs had higher concordance with classifications based on human data than classifications based on LLNA data. When borderline results were eliminated, concordances changed little (**Error! Reference source not found.**B). The most notable change was the decrease in the concordance of h-CLAT with both LLNA and human hazard classifications.

The concordances of potency classifications are summarized in

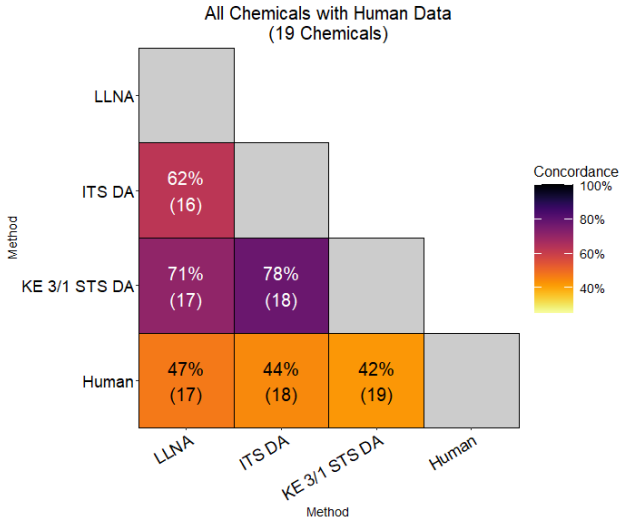


Figure 15. The concordance of the ITSv2 DA with the KE 3/1 STS DA (78%) was higher than the concordance of either DA with the LLNA (62% -71%) or with human data (42%-44%). The concordance of the LLNA potency classifications with human potency classifications was 47%,

and thus slightly higher than the concordances of the ITSv2 DA and the KE 3/1 STS DA with human potency classifications.

Table 23. Summary of Hazard and Potency Classification Results for 24 Substances with Human Data

Method	Unique Number Evaluated	Initial Number of INC or NA	Final Number of BL, INC, or NA[a]	Number Positive	Number Negative
DPRA Hazard	24	0	4	15	5
KS Hazard	24	1	1	17	6
h-CLAT Hazard	24	0	3	18	3
QSAR TBv4.5 Hazard	24	—	4	14 (14)[b]	6 (4)[b]
In Vivo GHS Hazard	24	—	2	17	5
In Vivo GHS Potency	24	—	3	14 (1A:6, 1B:8)	7
2o3 Hazard	24	0	3	18	3
ITSv2 Hazard	24	—	0	20	4
ITSv2 GHS Potency	24	—	1	19 (1A:6, 1B:13)	4
STS Hazard	24	—	0	21	3
STS GHS Potency	24	—	0	21 (1A:8, 1B:13)	3
In Vivo Human Potency	24	—	5	7 (1A:5, 1B:2)	12

Dashes (—) indicate the parameter is not relevant for that test method. Abbreviations: BL = borderline; INC = inconclusive; NA = not applicable or available.

Colors indicate the different types of assays/reference data: Yellow = in chemico/in vitro, Blue = in silico, Green = reference data, Pink = DAs.

[a] After the borderline evaluation of the individual test methods (DPRA, KeratinoSens and h-CLAT).

[b] Number in parentheses = number of in-domain predictions.

Table 24. Hazard and Potency Classification Results for 24 Substances with Human Data

BRTIV Number	Agency	DPRA Hazard	KS Hazard	h-CLAT Hazard	QSAR TBv.4.5 Hazard	In Vivo Hazard	In Vivo Potency	Human Hazard	Human Potency	2o3 Hazard	ITSv2 Hazard	ITSv2 Potency	STS Hazard	STS Potency
BRTIV-6	DTT	BL / 0	0	1	1	1	1B	0	NC	INC / 0	1	1B	1	1B
BRTIV-8	DTT	0	0	0	0	0	NC	0	NC	0	0	NC	0	NC
BRTIV-11[a]	DTT	1	1	1	1	1	1A	1	NA	1	1	1A	1	1A
BRTIV-17	DTT	1	1	1	1	1	1A	1	1B	1	1	1B	1	1B
BRTIV-24	DTT	1	1	1	1	1	1A	1	1A	1	1	1A	1	1A
BRTIV-30[b]	DTT	0	1	1	NA	1	1A	1	1A	1	1	1B	1	1A
BRTIV-34	DTT	1	1	1	1	1	1B	0	NC	1	1	1A	1	1B
BRTIV-36	DTT	1	1	1	1	1	1B	1	1B	1	1	1B	1	1B
BRTIV-38	DTT	1	1	1	1	1	1A	1	NA	1	1	1A	1	1A
BRTIV-40	DTT	1	1	1	1	1	1A	1	1A	1	1	1A	1	1A
BRTIV-63	EPA OPPT	1	1	1	0	0	NC	0	NC	1	1	1B	1	1B
BRTIV-64[a]	CPSC	1	1	1	1	1	NA	1	NA	1	1	1A	1	1A
BRTIV-68	CPSC	BL / 1	0	1	0	1	NA	0	NC	INC / 1	1	1B	1	1A
BRTIV-90	DTT	BL / 0	1	1	0	ND	ND	0	NC	1	1	1B	1	1B
BRTIV-97[c]	CPSC	1	INC	1	1	1	1B	0	NC	1	1	1B	1	1B
BRTIV-100[d]	CPSC	1	1	BL / 1	NA	1	1B	0	NC	1	1	INC	1	1B
BRTIV-106	EPA OPPT	0	0	0	0	0	NC	0	NC	0	0	NC	0	NC
BRTIV-110	EPA OPP	BL / 0	0	1	0	0	NC	0	NC	INC / 0	0	NC	1	1B
BRTIV-111	DTT	1	1	1	1	1	1B	0	NC	1	1	1B	1	1B
BRTIV-119	EPA OPP	1	1	BL / 1	1	0	NC	1	1A	1	1	1B	1	1B
BRTIV-135	EPA CCTE	0	0	0	1	1	1B	0	NC	0	0	NC	0	NC
BRTIV-142	CPSC	1	1	INC / 1	1	1	1B	1	1A	1	1	1B	1	1B

BRTIV-157[a,d]	EPA OPPT	0	1	1	NA	1	NA	1	NA	1	1	1B	1	1A
BRTIV-191[e,f]	FDA	1	1	0	NA	ND	ND	1	NA	1	1	1B	1	1B

Abbreviations: BL = borderline; INC = inconclusive; NA = not applicable or available; NC= not classified; 1 = positive; 0 = negative.

Colors indicate the different types of assays/reference data: Yellow = in chemico/in vitro, Blue = in silico, Green = reference data, Pink = DAs.

[a] Data came from tests on workers, patients, or previously sensitized subjects.

[b] QSAR hazard could not be predicted because substance was an inorganic compound.

[c] KeratinoSens results were inconclusive because, while there was no positive response observed, test substance solubility was too low to allow testing at concentrations specified in the test guideline.

[d] QSAR hazard could not be predicted because substance did not have a definitive chemical structure.

[e] Data came from a drug information package for METVIXIA.

[f] QSAR hazard could not be predicted because there was an insufficient number of similar chemicals with skin sensitization data.

3.7.1. Concordance of NAMs and In Vivo Data for Nominated Substances

A. **B.**

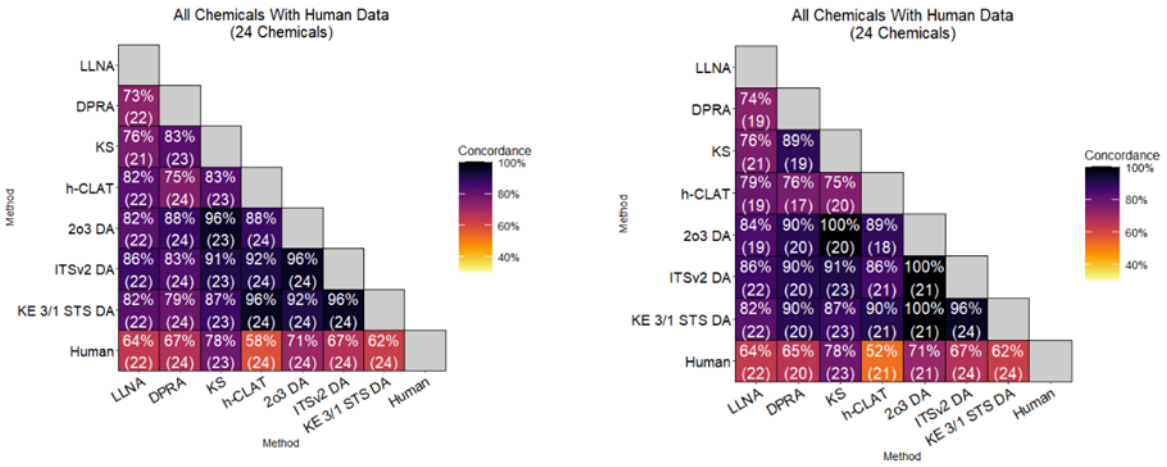


Figure 15. Concordance of Skin Sensitization Hazard Classifications for NAMs, LLNA, and Human Data.

15A shows concordance of all applicable results while 15B shows the concordance following application of borderline exclusion criteria to individual assays and the 2o3 DA. Inconclusive results are not shown.

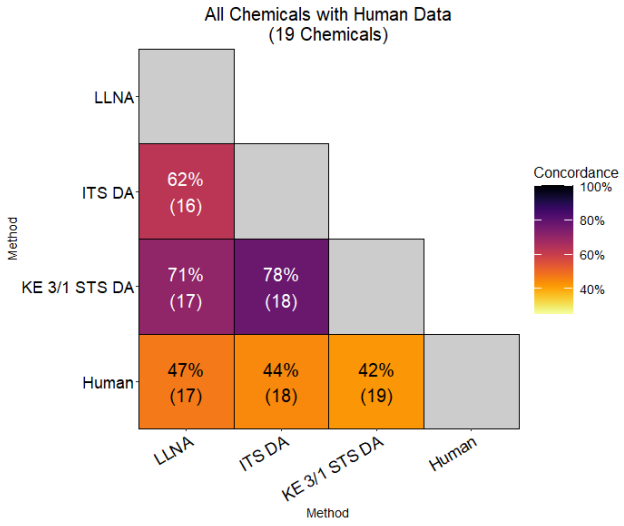


Figure 15. Concordance of Skin Sensitization Potency Classifications for DAs, LLNA, and Human Data.

4. SUMMARY

In this project, we evaluated the performance of individual in vitro methods and DAs for determining skin sensitization potential of a group of test substance nominated by six U.S. federal agencies and offices. We did this by comparing hazard and potency classifications based on data from these methods to those based on LLNA and human data. Sufficient LLNA data were available such that separate comparisons of classifications based on LLNA and data from the in vitro methods and DAs could be made for substances nominated by five groups (DTT, CPSC, EPA CCTE, EPA OPP, and EPA OPPT; described in Sections 3.1 through 3.6). Because human data were available for only 24 substances, the comparisons between classifications based on these data and data from NAMs and in vivo animal tests were made as a single group (Section 3.7). The performance on the non-animal methods for FDA are not compared to the others because only two substances were nominated by that agency.

Figure 16 summarizes the concordances of skin sensitization hazard classifications (sensitizer vs. nonsensitizer) based on NAMs data (individual in vitro methods and DAs) with those based on LLNA data, with tested substances grouped by nominator. Grouping substances by nominator is of interest because of the possibility that substances of interest to a particular group (for example, pesticides for EPA OPP) may have chemical characteristics in common that may affect their performance in a certain test method or DA. These reflect results after eliminating borderline tests for DPRA, KeratinoSens, and h-CLAT. These classifications are expected to have more certainty than classifications based on results before eliminating borderline tests, although excluding borderline results also reduces the number of analyzable substances. The NAMs had the highest concordance with the LLNA for the CPSC-nominated substances and the lowest concordance with the LLNA for the EPA OPP-nominated substances. DAs had higher concordance with the LLNA than the individual NAMs for CPSC-nominated and EPA CCTE-nominated substances. KeratinoSens had the highest concordance with the LLNA for the EPA OPPT- and EPA OPP-nominated substances. KeratinoSens also had higher concordance with animal data for agrochemicals skin sensitization assessments (Strickland et al. 2022).

Table 25 summarizes characteristics of each testing group that we evaluated to explore potential associations with inconclusive calls. Substances nominated by EPA OPP suggested that inconclusive calls may be correlated with substances being tested at starting concentrations below the recommended level. The EPA OPP-nominated substances also had the largest proportion of mixtures, which may have affected how well the individual tests performed. DPRA precipitates did not seem to correlate with inconclusive calls (Table 25).

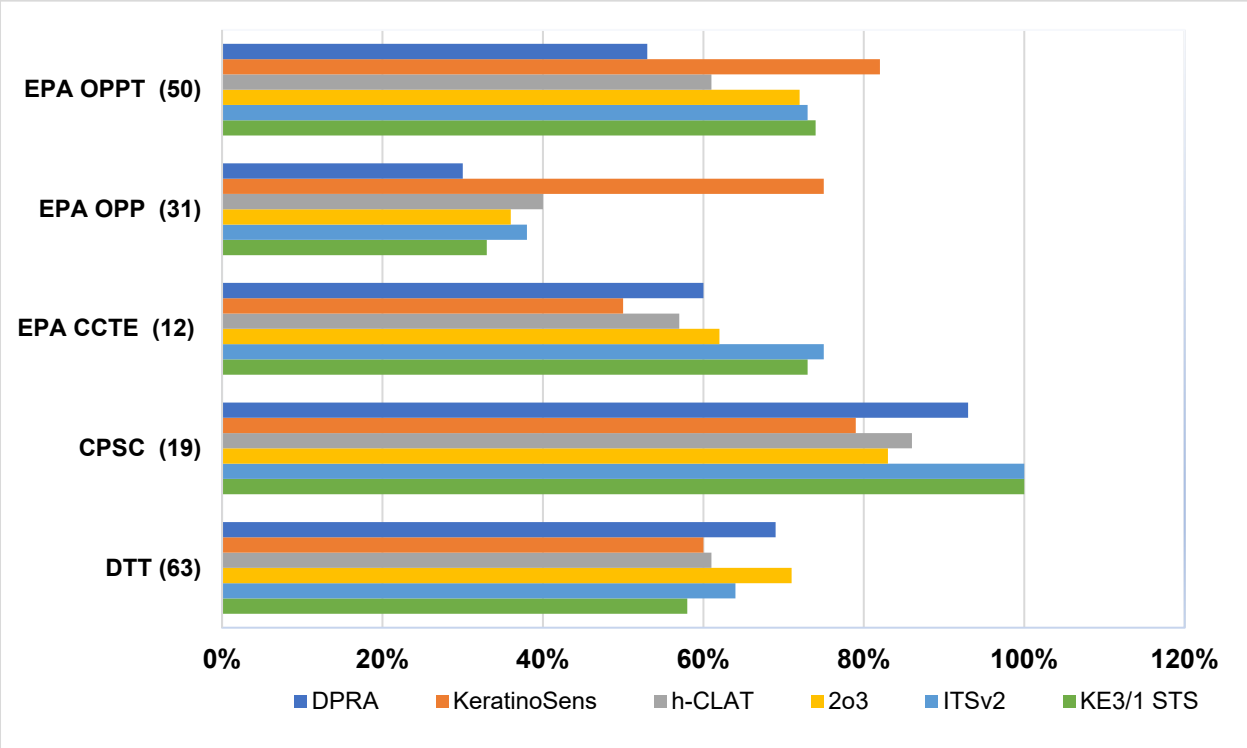


Figure 16. LLNA:NAM Concordance for Skin Sensitization Hazard Classifications. Concordance evaluation shown applies to results after borderline and inconclusive results were eliminated.

Table 25. Select Characteristics of Substances by Nominating Agency

Characteristic (numbers of)	DTT	FDA	CPSC	EPA CCTE	EPA OPP	EPA OPPT
Substances tested	63	2	22	12	31	50
Mixtures (including isomers)	2	0	2	0	22	2
Inorganic compounds	2	0	2	0	0	3
Metal compounds	2	0	5	0	1	7
Insoluble Chemicals (tested at lower than recommended concentration)	16 (KS); 5 (h-CLAT)	0	13 (KS); 1 (h-CLAT)	6 (KS)	3 (DPRA); 11 (KS); 3 (h-CLAT)	3 (DPRA); 23 (KS); 9 (h-CLAT)
DPRA precipitates	20	0	11	6	2	18
Inconclusive in individual test method(s)	2 (KS)	0	3 (DPRA); 1 (KS)	0	1 (DPRA); 9 (KS)	1 (DPRA); 4 (KS)

5. CONCLUSIONS

To evaluate NAMs applicability for assessing skin sensitization potential, NICEATM tested substance nominated by U.S. federal agencies and offices in individual in vitro test methods and DAs. Nominated substances were used to: 1) characterize skin sensitization hazard using in vitro methods; 2) compare hazard classifications based on in vitro test outcomes to one another and to those based on in vivo data; 3) use in vitro results as inputs into DAs accepted for hazard and potency classification; 4) compare the classifications predicted by the DAs to classifications

based on in vivo data; and 5) consider all classification results with respect to individual agency remits.

For hazard classification, concordances of the classifications based on data from the in vitro test methods were higher among the different methods than between classifications based on data from these methods and classifications based on data from the LLNA. Similarly, concordances among the classifications based on DAs were higher than between the classifications based on DAs and the classifications based on data from LLNA. Concordances between classifications based on data from the DAs and those based on human data, where available, were similar to and sometimes better than LLNA concordance with human data. This suggests that the DAs may be overall better predictors of human sensitization hazard and potency than the LLNA. Another factor that may have contributed to the lower concordance between classifications from DAs and classifications based on LLNA data is specific to formulations. The compositions of the formulations used for the LLNA testing may have differed from those used for testing in the in vitro methods because the formulations tested in the in vitro methods were commercial off-the-shelf products, whereas the formulations tested in the LLNA may have been pre-market products.

Overall, this study suggests that in vitro testing and applying DAs can be a useful alternative to animal testing for some federal agency programs. However, as discussed in the Summary, results from the NAMs varied by the types of substances nominated and some substances were not compatible with these test systems (Table 25). This particularly applies to substances nominated by OPP, where limited solubility resulted in limited success in using different NAMs. This study highlights that the methods within a DA can be used to predict skin sensitization hazard for substances and formulations beyond what have been previously considered in support of the OECD test guideline, although further assessment may be warranted.

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Appendix A: In Vitro Results, In Silico Data, Physicochemical Data, In Vivo Reference Data, and Defined Approach Results

https://ntp.niehs.nih.gov/whatwestudy/niceatm/test-method-evaluations/skin-sens/da

Appendix B: BRT In Vitro Testing Results

https://doi.org/10.22427/NICEATM-DATA-NICEATM-05