Leontaridou et al.:

The Borderline Range of Toxicological Methods: Quantification and Implications for Evaluating Precision

Supplementary Data

Appendix 1: Set of substances used in the borderline range calculation

Where substance names could not be published for reasons of data confidentiality, we numbered them consecutively.

Tab. S1.1: Substances in the set for calculating the BR of the DPRA prediction model

Substance	No. of runs	Test substance concentrations considered (mM)	Study year
Ethylene glycol dimethacrylate* (positive control)	211	0.5, 1, 5, 10, 50, 100	2014
Substance 1	24	1, 10, 100	2015
Substance 2	6	100	2015
Substance 3	3	100	2015
Substance 4	12	1, 5, 10, 100	2014
Substance 5	9	1, 5, 10	2014
Substance 6	12	1, 5, 10, 100	2014
Substance 7	12	1, 5, 10, 100	2014
Substance 8	3	100	2014
Substance 9	6	1, 10, 100	2015
Substance 10	6	1, 10, 100	2015
Substance 11	6	1, 10, 100	2015
Substance 12	4	1, 10	2015
Substance 13	6	1, 10, 100	2015
Substance 14	6	1, 10, 100	2015
Substance 15	4	1, 10	2015
Substance 16	4	1, 10	2015
Substance 17	6	1, 10, 100	2015
Substance 18	6	1, 10, 100	2015
Substance 19	4	1, 10	2014
Substance 20	6	1, 10, 100	2014
Substance 21	6	1, 10, 100	2014
Substance 22	6	1, 10, 100	2014
Substance 23	6	1, 10, 100	2014
Substance 24	6	1, 10, 100	2014
Substance 25	6	1, 10, 100	2014
Substance 26	6	1, 10, 100	2014
Substance 27	6	1, 10, 100	2014
Substance 28	6	1, 10, 100	2014
Substance 29	6	1, 10, 100	2014
Substance 30	3	100	2014
Substance 31	3	100	2014
Substance 32	3	100	2014
Substance 33	3	100	2014
Substance 34	3	100	2013
Substance 35	3	3.76%	2013
Substance 36	3	3.76%	2013
Substance 37	3	100	2013
Substance 38	3	100	2013
Substance 39	3	100	2013
Substance 40	3	100	2012
Substance 41	3	100	2013

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Tab. S1.2: Substances in the set for calculating the BR of the LuSens prediction model

Substance	No. of runs	Test substance range of concentrations considered (µg/ml)	Study year
Lactic acid (negative control)	395	450	2013-2015
Substance 1	36	1.4 - 5.1	2013-2015
Substance 2	53	0.15 - 0.54	2013-2015
Substance 3	48	3.9 - 14.0	2013-2015
Substance 4	144	0.06 - 0.92	2013-2015
Substance 5	174	0.65 - 16.8	2013-2015
Substance 6	96	77.0 - 479.0	2013-2015
Substance 7	96	0.03 - 0.53	2013-2015
Substance 8	96	0.31 - 4.75	2013-2015
Substance 9	48	4.5 - 16.2	2013-2015
Substance 10	48	4.7 - 42.9	2013-2015
Substance 11	120	4.0 - 176.0	2013-2015
Substance 12	48	6.0 - 46.0	2013-2015
Substance 13	72	3.9 - 20.2	2013-2015
Substance 14	48	4.6 - 16.5	2013-2015
Substance 15	48	0.44 - 1.57	2013-2015
Substance 16	48	1283.0 - 7942.0	2013-2015
Substance 17	48	636.0 - 2278.0	2013-2015
Substance 18	48	14.0 - 85.0	2013-2015
Substance 19	48	226.0 - 2012.0	2013-2015
Substance 20	36	0.59 - 2.02	2013-2015
Substance 21	72	0.91 - 3.27	2013-2015
Substance 22	96	6.9 - 106.0	2013-2015
Substance 23	96	1.8 - 28.0	2013-2015
Substance 24	72	542.0 - 1941.0	2013-2015
Substance 25	72	450.0 - 2000.0	2013-2015

Tab. S1.3: Substances in the set for calculating the BR of the h-CLAT prediction model

Substance	No. of runs	Test substance concentrations considered (µg/ml)	Study year
Lactic acid (negative control)	53	1000	2013-2015
DNCB (positive control)	53	4.0	2013-2015
Substance 1	32	7396.0 - 2064.0	2013-2015
Substance 2	30	5655.0 - 29176.0	2013-2015
Substance 3	40	12.3 - 64.0	2013-2015
Substance 4	32	69.0 - 510.0	2013-2015
Substance 5	48	14.2 - 73.4	2013-2015
Substance 6	48	2.2 - 8.0	2013-2015
Substance 7	32	29.0 - 105.0	2013-2015
Substance 8	48	1589.0 - 5695.0	2013-2015
Substance 9	48	13.0 - 48.0	2013-2015
Substance 10	32	115.0 - 710.0	2013-2015
Substance 11	32	14.0 - 51.0	2013-2015

Tab. S1.4: Substances in the set for calculating the BR of the LLNA prediction model

Substance	CAS no.	No. of runs	Test substance concentrations considered (µg/ml)	Study year
DL-Lactic acid	50-21-5	20	5, 10, 25	2013-2015
Salicylic acid	69-72-7	20	5, 10, 25	2013-2015
Chlorobenzene	108-90-7	20	25, 50, 100	2013-2015
Methyl methacrylate	80-62-6	20	25, 50, 100	2013-2015
2-Mercaptobenzothiazole	149-30-4	19	0.75, 2.0, 7.5	2013-2015
Methyl salicylate	119-36-8	20	10, 25, 50	2013-2015
MCI / MI	26172-55-4 & 2682-20-4	40	0.005, 0.05, 0.2, 0.1, 0.5, 1	2013-2015
Sodium dodecyl sulfate	151-21-3	20	1, 5, 10	2013-2015
Imidazolidinyl urea	39236-46-9	20	10, 25, 50	2013-2015
Ethylenglycolmethacrylate (EGDMA)	97-90-5	20	25, 50, 100	2013-2015
Nickel(II) chloride	7718-54-9	20	1, 2.5, 5	2013-2015
Cinnamic alcohol	104-54-1	20	10, 25, 50	2013-2015
Isopropanol	67-63-0	20	25, 50, 100	2013-2015
Phenylbenzoate	93-99-2	40	5,10, 15, 25, 40	2013-2015
Isoeugenol	97-54-1	20	1, 5, 10	2013-2015
Xylene	1330-20-7	20	25, 50, 100	2013-2015
Alpha-Hexylcinnamaldehyde	101-86-0	20	5, 10, 25	2013-2015
p-Phenylenediamine	106-50-3	20	0.05, 0.1, 0.5	2013-2015
Citral	5392-40-5	20	5, 10, 25	2013-2015
Cobalt(II) chloride	7646-79-9	20	0.25, 0.5, 1	2013-2015
Eugenol	97-53-0	20	2.5, 10, 25	2013-2015
1-Chloro-2,4-dinitrobenzene (DNCB)	97-00-7	20	0.025, 0.1, 0.25	2013-2015

Appendix 2: A numerical illustration of the approach for quantifying the pooled standard deviation and the BR

The following example illustrates how the approach described for determining the BR of a testing method's prediction model is applied. The example uses results from substances tested with LuSens. For confidentiality reasons the substances and concentrations tested are anonymised.

Suppose that the experimental set consists of two substances. Following Ramirez et al. (2014), each substance is tested in six concentrations, and each concentration is tested three times per substance. Thus, results from three replicates are available per substance and concentration. The classification threshold of LuSens is the luciferase fold induction T=1.5.

Using the notation introduced in Section 2.2, the dataset for calculating the pooled standard deviation SD_p and the corresponding BR is as follows:

Tab. S2.1: Notation related to the illustrative case

Notation	Explanation
T	1.5 FI
i	Substance (i=1,2)
n	Number of substances tested (n=2)
j	Concentration tested per substance i (j=1,2,3,4,5,6)
k_i	Number of concentrations per substance (k_i=6)
$r_{i,j}$	Number of replicates per substance i and concentration j ($r_{i,j}$=3)
l	Replicate per substance i and concentration j (l=1,2,3)
$y_{i,j,l}$	Test result of substance i, concentration j and replicate l
$\bar{y}_{i,j}$	Arithmetic mean of test results for substance i and concentration j

Table S2.2 below shows the experimental results revealed, the arithmetic means of test results and the standard deviation of results per substance and concentration calculated according to Eq. (3):

Tab. S2.2: Experimental results and standard deviation of results per substance and concentration

Substance i	Concentration j	Replicate l	Test result $y_{i,j,l}$	Mean test result $\bar{y}_{i,j}$	Sample variance $\sigma^2_{i,j}$	Weighted variance per substance and concentration $(r_{i,j}-1)\sigma^2_{i,j}$
1	1	1 2 3	1.39 0.88 0.98	1.083	0.073	0.15
1	2	1 2 3	0.96 1.02 1.01	0.996	0.001	0.00
1	3	1 2 3	1.35 0.96 1.11	1.14	0.039	0.08
1	4	1 2 3	1.43 1.16 0.93	1.173	0.063	0.13
1	5	1 2 3	1.29 0.86 1.18	1.11	0.050	0.10
1	6	1 2 3	1.18 1.24 1.28	1.23	0.003	0.01
2	1	1 2 3	3.58 2.97 3.12	3.23	0.101	0.20
2	2	1 2 3	4.01 3.22 3.06	3.43	0.259	0.52
2	3	1 2 3	5.62 3.89 3.22	4.24	1.534	3.07
2	4	1 2 3	6.76 4.74 3.45	4.98	2.783	5.57
2	5	1 2 3	5.41 5.10 3.78	4.76	0.749	1.50
2	6	1 2 3	8.45 8.28 5.72	7.48	2.339	4.68

Source: BASF (2015)

According to Eq. (2), the pooled standard deviation of the experimental sample is

$$SD_p = \sqrt{\frac{\sum_{i=1}^{n}\sum_{j=1}^{k_i}(r_{i,j}-1)*\sigma_{i,j}^2}{\sum_{i=1}^{n}\sum_{j=1}^{k_i}(r_{i,j}-1)}} .$$

Thus, the SD_p of the dataset shown in Table S2.2 is 0.82.
Following Eq. (3), the BR around the classification threshold is
$BR=\{T+SD_p, T-SD_p\}=\{1.5-0.82, 1.5+0.82\}=\{0.68, 2.32\}$.

Appendix 3: Experimental substance sets used for identifying borderline substances

Tab. S3.1: Results from applying different borderline ranges to the experimental set of substances tested with the DPRA (results compared to the LLNA)[1]

Substance	Cas no.	*SDp* = 1.52 (BR = 4.86% - 7.90%)	*SDp* = 3.49 (BR = 2.89% - 9.87%)	*SDp* = 5.03 (BR = 1.35% - 11.41%)
Benzalkonium chloride	8001-54-5	TN	TN	N
Lactic acid	50-21-5	TN	TN	N
Octanenitrile	124-12-9	TN	TN	N
Undec-10-enal	112-45-8	FN	FN	N
Benzyl benzoate	120-51-4	FN	FN	N
Methyl 4-hydroxybenzoate (Methylparaben)	99-76-3	TN	TN	N
Butylbenzylphthalate	85-68-7	TN	TN	N
4-Hydroxybenzoic acid	99-96-7	TN	TN	N
Sulfanilamide	63-74-1	TN	TN	N
Cocamidopropyl betaine	61789-40-0	TN	TN	N
Benzene,1-methoxy-4-methyl-2-nitro (4-Methyl-2-nitroanisole)	119-10-8	TN	TN	N
Squaric acid diethyl ester	5231-87-8	FN	FN	N
Clofibrate (Ethyl (2-(4-chlorophenoxy)-2-methylpropanoate)	637-07-0	TN	TN	N
α-Amyl cinnamic aldehyde	122-40-7	FN	FN	N
Streptomycin sulfate	3810-74-0	TN	TN	N
α-iso-Methylionone	127-51-5	FN	FN	N
Carbonic acid, dioctyl ester	1680-31-5	TN	TN	N
Hexyl salicylate	6259-76-3	FN	FN	N
Benzyl cinnamate	103-41-3	FN	FN	N
Benzyl salicylate	118-58-1	FN	FN	N
Sulfanilic acid	121-57-3	TN	N	N
Isopropyl myristate a	110-27-0	FN	N	N
p-Aminobenzoic acid	150-13-0	TN	N	N
Tartaric acid	87-69-4	TN	N	N
Zinc sulfate	7733-02-0	TN	N	N
Dioctyl ether	629-82-3	TN	N	N
2,2-Azobis phenol	2050-14-8	FN	N	N
Benzaldehyde	100-52-7	TN	N	N
Farnesol	4602-84-0	FN	N	N
3-Aminophenol	591-27-5	FN	N	N
(+/-) Linalool	78-70-6	FN	N	N
Diethylenetriamine	111-40-0	FN	N	N

[1] Classifications revealed from Urbisch et al. (2015).

Substance	Cas no.	*SDp* = 1.52 (BR = 4.86% - 7.90%)	*SDp* = 3.49 (BR = 2.89% - 9.87%)	*SDp* = 5.03 (BR = 1.35% - 11.41%)
Octanoic acid, 4-methyl-2-pentylbutyl ester	868839-23-0	TN	N	N
Salicylic acid	69-72-7	N	N	N
Geraniol	106-24-1	N	N	N
Benzyl alcohol	100-51-6	N	N	N
Tween 80	9005-65-6	N	N	N
3-Dimethylamino propylamine	109-55-7	N	N	N
cis-6-Nonenal	2277-19-2	N	N	N
Ethyl vanillin	121-32-4	N	N	N
Undecylenic acid	112-38-9	N	N	N
2-methoxy-4-methylphenol	93-51-6	N	N	N
Ethyl benzoylacetate	94-02-0	N	N	N
Dihydroeugenol (2-methoxy-4-propyl-phenol)	2785-87-7	N	N	N
α-Hexyl cinnamic aldehyde	101-86-0	N	N	N
N,N-Diethyl-m-toluanimde	134-62-3	P	P	P
Penicillin G	61-33-6	P	P	P
d,l-Citronellol	106-22-9	P	P	P
Pentachlorophenol	87-86-5	P	P	P
p-tert-Butyl-alpha-ethyl hydrocinnamal (Lilial)	80-54-6	P	P	P
1-Bromobutane	109-65-9	P	P	P
Fumaric acid	110-17-8	P	P	P
Glucose	50-99-7	P	P	P
R(+)-Limonene	5989-27-5	TP	P	P
Ethylenediamine free base	107-15-3	TP	P	P
Vanillin	121-33-5	FP	FP	N
Cyclamen aldehyde	103-95-7	TP	TP	P
Propyl paraben	94-13-3	TN	TN	TN
4-Methoxyacetophenone (Acetanisole)	100-06-1	TN	TN	TN
6-Methylcoumarin	92-48-8	TN	TN	TN
Nonanoic acid	112-05-0	FN	FN	FN
Isopropanol	67-63-0	FN	FN	FN
Methyl salicylate	119-36-8	TN	TN	TN
Dibutyl phthalate	84-74-2	TN	TN	TN
Pyridine	110-86-1	TN	TN	TN
dl-α-Tocopherol	10191-41-0	TN	TN	TN
Clotrimazole	23593-75-1	FN	FN	FN
Methyl pyruvate	600-22-6	FN	FN	FN
1-Butanol	71-36-3	TN	TN	TN

Substance	Cas no.	*SDp* = 1.52 (BR = 4.86% - 7.90%)	*SDp* = 3.49 (BR = 2.89% - 9.87%)	*SDp* = 5.03 (BR = 1.35% - 11.41%)
Xylene	1330-20-7	FN	FN	FN
Diethyl phthalate	84-66-2	TN	TN	TN
Vinylidene dichloride	75-35-4	TN	TN	TN
Oxalic acid anhydrous	144-62-7	FN	FN	FN
Octanoic acid (Caprylic acid)	124-07-2	TN	TN	TN
Coumarin	91-64-5	TN	TN	TN
Dimethyl formamide	68-12-2	TN	TN	TN
Glycerol	56-81-5	TN	TN	TN
2,2,6,6-Tetramethyl-3,5-heptanedione	1118-71-4	FN	FN	FN
N,N-Dibutylaniline	613-29-6	FN	FN	FN
Resorcinol	108-46-3	FN	FN	FN
Chlorobenzene	108-90-7	TN	TN	TN
Propylene glycol (1,2-Propanediol)	57-55-6	TN	TN	TN
4-Chloroaniline	106-47-8	FN	FN	FN
7,12-Dimethylbenz[α]anthracene	57-97-6	FN	FN	FN
Aniline	62-53-3	FN	FN	FN
Saccharin	81-07-2	TN	TN	TN
Hexadecyltrimethylammonium bromide (Cetrimide)	57-09-0	TN	TN	TN
n-Hexane	110-54-3	TN	TN	TN
Tropolone	533-75-5	TP	TP	TP
Cinnamyl Alcohol	104-54-1	TP	TP	TP
R-Carvone	6485-40-1	TP	TP	TP
Benzocaine	94-09-7	FP	FP	FP
3-Phenoxypropiononitrile	3055-86-5	FP	FP	FP
2-Acetyl-cyclohexanone	874-23-7	FP	FP	FP
Diethyl sulfate	64-67-5	TP	TP	TP
2-Phenylpropionaldehyde	93-53-8	TP	TP	TP
5-Methyl-2,3-hexanedione	13706-86-0	TP	TP	TP
1-Iodohexane	638-45-9	FP	FP	FP
2,2-Bis-[4-(2-hydroxy-3-methacryloxypropoxy)phenyl)]-propane (Bis-GMA)	1565-94-2	TP	TP	TP
Farnesal	502-67-0	TP	TP	TP
α-methyl-trans-Cinnamaldehyde	101-39-3	TP	TP	TP
3,4-Dihydrocoumarin	119-84-6	TP	TP	TP
Eugenol	97-53-0	TP	TP	TP
Lyral / 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde	31906-04-4	TP	TP	TP
Nickel chloride	7718-54-9	FP	FP	FP

Substance	Cas no.	*SDp* = 1.52 (BR = 4.86% - 7.90%)	*SDp* = 3.49 (BR = 2.89% - 9.87%)	*SDp* = 5.03 (BR = 1.35% - 11.41%)
Bisphenol A-diglycidyl ether	1675-54-3	TP	TP	TP
1,2,4-Benzenetricarboxylic anhydride (Trimellitic anhydride)	552-30-7	TP	TP	TP
1-(p-Methoxyphenyl)-1-penten-3-one	104-27-8	TP	TP	TP
3-Propylidenephthalide	17369-59-4	TP	TP	TP
Perillaldehyde	2111-75-3	TP	TP	TP
Tetrachloro-salicylanilide	1154-59-2	TP	TP	TP
2-Fluoro-5-nitroaniline	369-36-8	FP	FP	FP
Phthalic anhydride	85-44-9	TP	TP	TP
1,2-cyclohexane dicarboxylic anhydride	85-42-7	TP	TP	TP
Squaric acid	2892-51-5	TP	TP	TP
Formaldehyde	50-00-0	TP	TP	TP
2-Hydroxypropyl methacrylate	923-26-2	FP	FP	FP
1-Phenyl-1,2-propanedione	579-07-7	TP	TP	TP
Cobalt chloride	7646-79-9	TP	TP	TP
Methylmethacrylate	80-62-6	TP	TP	TP
Phenyl benzoate	93-99-2	TP	TP	TP
3-Chloro-4-methoxybenzaldehyde (3-Chloro-p-anisaldehyde)	4903-09-7	FP	FP	FP
Butyl glycidyl ether	2426-08-6	TP	TP	TP
Imidazolidinyl urea	39236-46-9	TP	TP	TP
1-Naphthol	90-15-3	TP	TP	TP
Ethanol-2-butoxy acetate	112-07-2	FP	FP	FP
1-Bromohexane	111-25-1	TP	TP	TP
Phenylacetaldehyde	122-78-1	TP	TP	TP
Benzoic acid	65-85-0	FP	FP	FP
1-Iodohexadecane	544-77-4	TP	TP	TP
Citral	5392-40-5	TP	TP	TP
Bandrowski's Base (N,N-bis(4-aminophenyl)-2,5-diamino-1,4-quinone-diimine)	20048-27-5	TP	TP	TP
1,1,3-Trimethyl-2-formylcyclohexa-2,4-diene (Safranal)	116-26-7	TP	TP	TP
4-Vinyl pyridine	100-43-6	TP	TP	TP
Benzylidene acetone (4-Phenyl-3-buten-2-one)	122-57-6	TP	TP	TP
2-Nitro-1,4-phenylendiamine	5307-14-2	TP	TP	TP
2,5-Diaminotoluene sulfate (PTD)	615-50-9	TP	TP	TP
Hydroxycitronellal	107-75-5	TP	TP	TP
MCI/MI	26172-55-4 & 2682-20-4	TP	TP	TP
Sodium lauryl sulfate / sodium dodecyl sulfate (SDS)	151-21-3	TP	TP	TP

Substance	Cas no.	*SDp* = 1.52 (BR = 4.86% - 7.90%)	*SDp* = 3.49 (BR = 2.89% - 9.87%)	*SDp* = 5.03 (BR = 1.35% - 11.41%)
Methyl-2-octynoate / Methyl heptine carbonate	111-12-6	TP	TP	TP
2-Methyl-2H-Isothiazol-3-one (MI)	2682-20-4	TP	TP	TP
4-Allylanisole	140-67-0	TP	TP	TP
Diphenylcyclopropenone	886-38-4	TP	TP	TP
Lauryl gallate	1166-52-5	TP	TP	TP
Iodopropynyl butylcarbamate	55406-53-6	TP	TP	TP
Furil	492-94-4	FP	FP	FP
2-Methylundecanal	110-41-8	TP	TP	TP
N,N-dimethyl-4-nitrosoaniline	138-89-6	TP	TP	TP
2-Propylheptyl acrylate	149021-58-9	TP	TP	TP
trans-2-Hexenal	6728-26-3	TP	TP	TP
5-Amino-2-methylphenol	2835-95-2	TP	TP	TP
Chlorothalonil	1897-45-6	TP	TP	TP
2-Mercaptobenzothiazole	149-30-4	TP	TP	TP
Methyl 2-nonynoate	111-80-8	TP	TP	TP
Methyl methanesulphonate	66-27-3	TP	TP	TP
4-(N-Ethyl-N-2-methan-sulphonamido-ethyl)-2-methyl-1,4-phenylenediamine (CD3)	25646-71-3	TP	TP	TP
1,2-Dibromo-2,4-dicyanobutane (MDGN, Methyldibromo glutaronitrile)	35691-65-7	TP	TP	TP
trans-2-Decenal	3913-71-1	TP	TP	TP
Tetramethylthiuram disulfide	137-26-8	TP	TP	TP
1,2-Benzisothiazolin-3-one (Proxel active)	2634-33-5	TP	TP	TP
Propanoic acid, 3-bromo-methyl ester (Methyl-3-bromopropionate)	3395-91-3	FP	FP	FP
4-Carboxyphenylacetate	2345-34-8	TP	TP	TP
Cinnamic aldehyde	104-55-2	TP	TP	TP
2-Aminophenol	95-55-6	TP	TP	TP
Diethyl acetaldehyde	97-96-1	TP	TP	TP
Glutaraldehyde	111-30-8	TP	TP	TP
Abietic acid	514-10-3	TP	TP	TP
4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one (Oxazolone)	15646-46-5	TP	TP	TP
4-Amino-m-cresol	2835-99-6	TP	TP	TP
Isoeugenol	97-54-1	TP	TP	TP
2-Ethylhexyl acrylate	103-11-7	TP	TP	TP
2,4-Heptadienal	5910-85-0	TP	TP	TP
2,4-Dinitrobenzenesulfonic acid, sodium salt	885-62-1	TP	TP	TP

Substance	Cas no.	*SDp* = 1.52 (BR = 4.86% - 7.90%)	*SDp* = 3.49 (BR = 2.89% - 9.87%)	*SDp* = 5.03 (BR = 1.35% - 11.41%)
Benzyl bromide	100-39-0	TP	TP	TP
2,4,6-Trinitrobenzenesulfonic acid	2508-19-2	TP	TP	TP
Propyl gallate	121-79-9	TP	TP	TP
4-Nitrobenzyl bromide	100-11-8	TP	TP	TP
Glyoxal	107-22-2	TP	TP	TP
Ethylene glycol dimethacrylate (EGDMA)	97-90-5	TP	TP	TP
2,3-Butanedione	431-03-8	TP	TP	TP
Isophorone diisocyanate	4098-71-9	TP	TP	TP
5-Chloro-2-methyl-4-isothiazolin-3-one (MCI)	26172-55-4	TP	TP	TP
1,6-hexamethylene diisocyanate	822-06-0	TP	TP	TP
Hydroquinone	123-31-9	TP	TP	TP
Maleic anhydride	108-31-6	TP	TP	TP
1,4-Phenylenediamine	106-50-3	TP	TP	TP
4-(Methylamino)phenol sulfate (Metol)	55-55-0	TP	TP	TP
1-Chloro-2,4-dinitrobenzene (Dinitrochlorobenzene, DNCB)	97-00-7	TP	TP	TP
Fluorescein-5-isothiocyanate	3326-32-7	TP	TP	TP
3-Methylcatechol	488-17-5	TP	TP	TP
Diethyl maleate	141-05-9	TP	TP	TP
Benzoyl peroxide	94-36-0	TP	TP	TP
2-Hydroxyethyl acrylate	818-61-1	TP	TP	TP
Ethyl acrylate	140-88-5	TP	TP	TP
Methyl acrylate	96-33-3	TP	TP	TP
Butyl acrylate	141-32-2	TP	TP	TP
p-Benzoquinone	106-51-4	TP	TP	TP
Tosylchloramide sodium (Chloramine T)	127-65-1	TP	TP	TP

Tab. S3.2: Results from applying different borderline ranges to the experimental set of substances tested with the LuSens (results compared to the LLNA)[1]

Substance	Cas no.	SD_p = 0.23 (BR = 1.27-1.73)	SD_p = 0.24 (BR = 1.26-1.74)
1-Butanol	71-36-3	N	N
4-Allylanisole	140-67-0	P	P
Benzoyl peroxide	94-36-0	P	P
p-Benzoquinone	106-51-4	P	P
1,2-Dibromo-2,4-dicyanobutane (MDGN, Methyldibromo glutaronitrile)	35691-65-7	P	P
Imidazolidinyl urea	39236-46-9	TP	P
Phthalic anhydride	85-44-9	FN	FN
Resorcinol	108-46-3	FN	FN
Sodium lauryl sulfate / sodium dodecyl sulfate (SDS)	151-21-3	FN	FN
Nickel chloride	7718-54-9	TN	TN
Salicylic acid	69-72-7	TN	TN
Farnesal	502-67-0	FN	FN
Propyl gallate	121-79-9	FN	FN
Hexadecyltrimethylammonium bromide (Cetrimide)	57-09-0	TN	TN
Lactic acid	50-21-5	TN	TN
Aniline	62-53-3	FN	FN
4-Hydroxybenzoic acid	99-96-7	TN	TN
Glucose	50-99-7	TN	TN
Sulfanilamide	63-74-1	TN	TN
Penicillin G	61-33-6	FN	FN
p-Aminobenzoic acid	150-13-0	TN	TN
Ethylenediamine free base	107-15-3	FN	FN
Phenyl benzoate	93-99-2	FN	FN
Glycerol	56-81-5	TN	TN
Cocamidopropyl betaine	61789-40-0	TN	TN
Propylene glycol (1,2-Propanediol)	57-55-6	TN	TN
n-Hexane	110-54-3	TN	TN
Isopropanol	67-63-0	TN	TN
Fumaric acid	110-17-8	TN	TN
Tartaric acid	87-69-4	TN	TN
Xylene	1330-20-7	FN	FN
Pyridine	110-86-1	FN	FN
Vanillin	121-33-5	TN	TN
Octanoic acid, 4-methyl-2-pentylbutyl ester	868839-23-0	TN	TN
Benzyl alcohol	100-51-6	FP	FP
Dioctyl ether	629-82-3	FP	FP
Hydroxycitronellal	107-75-5	TP	TP
Methyl salicylate	119-36-8	FP	FP
1,6-hexamethylene diisocyanate	822-06-0	TP	TP

[1] Classifications revealed from Urbisch et al. (2015).

Substance	Cas no.	*SDp* = 0.23 (BR = 1.27-1.73)	*SDp* = 0.24 (BR = 1.26-1.74)
Potassium dichromate	7778-50-9	TP	TP
4-Nitrobenzyl bromide	100-11-8	TP	TP
α-Hexyl cinnamic aldehyde	101-86-0	TP	TP
1-Chloro-2,4-dinitrobenzene (Dinitrochlorobenzene, DNCB)	97-00-7	TP	TP
Diethyl phthalate	84-66-2	TN	TN
2-Ethylhexyl acrylate	103-11-7	TP	TP
2-Phenylpropionaldehyde	93-53-8	TP	TP
6-Methylcoumarin	92-48-8	FP	FP
Tween 80	9005-65-6	FP	FP
Propyl paraben (propyl-4-hydroxybenzoate)	94-13-3	FP	FP
Formaldehyde	50-00-0	TP	TP
Isophorone diisocyanate	4098-71-9	TP	TP
2-Propylheptyl acrylate	149021-58-9	TP	TP
Glyoxal	107-22-2	TP	TP
Ethyl acrylate	140-88-5	TP	TP
Butyl glycidyl ether	2426-08-6	TP	TP
Tetramethylthiuram disulfide	137-26-8	TP	TP
Eugenol	97-53-0	TP	TP
2,4,6-Trinitrobenzenesulfonic acid	2508-19-2	TP	TP
Glutaraldehyde	111-30-8	TP	TP
Methyl 4-hydroxybenzoate (Methylparaben)	99-76-3	FP	FP
MCI/MI	26172-55-4 & 2682-20-4	TP	TP
Cinnamyl Alcohol	104-54-1	TP	TP
Methylmethacrylate	80-62-6	TP	TP
Cobalt chloride	7646-79-9	TP	TP
4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one (Oxazolone)	15646-46-5	TP	TP
4-(Methylamino)phenol sulfate (Metol)	55-55-0	TP	TP
Undecylenic acid	112-38-9	TP	TP
2,3-Butanedione	431-03-8	TP	TP
4-Methoxyacetophenone (Acetanisole)	100-06-1	FP	FP
Butyl acrylate	141-32-2	TP	TP
1,4-Phenylenediamine	106-50-3	TP	TP
Methyl acrylate	96-33-3	TP	TP
Diethyl maleate	141-05-9	TP	TP
Benzylidene acetone (4-Phenyl-3-buten-2-one)	122-57-6	TP	TP
Cinnamic aldehyde	104-55-2	TP	TP
2-Mercaptobenzothiazole	149-30-4	TP	TP
Isoeugenol	97-54-1	TP	TP
Ethylene glycol dimethacrylate (EGDMA)	97-90-5	TP	TP
Citral	5392-40-5	TP	TP

Tab. S3.3: Results from applying different borderline ranges to the experimental set of substances tested with the h-CLAT (results compared to the LLNA)[1]

Substance	CAS no.	CD86 = (1.24-1.76) / CD54 = (1.81-2.19)	CD86 = (1.2-1.8) / CD54 = (1.74-2.26)
4-Phenylenediamine	106-50-3	P	P
Phenyl benzoate	93-99-2	P	P
Ethylene diamine	107-15-3	P	P
Aniline	62-53-3	P	P
Farnesal	502-67-0	P	P
Methyldibromo glutaraldehyde	35691-65-7	P	P
p-Benzoquinone	106-51-4	P	P
Propyl gallate	121-79-9	P	P
Citral	5392-40-5	TP	P
Cobalt chloride	7646-79-9	TP	P
MCI/MI	26172-55-4 & 2682-20-4	TP	TP
1-Chloro-2,4-dinitrobenzene	97-00-7	TP	TP
Cinnamic alcohol	104-54-1	TP	TP
Methyl methacrylate	80-62-6	TP	TP
Isopropanol	67-63-0	TN	TN
DL-Lactic acid	50-21-5	TN	TN
Methyl salicylate	119-36-8	TN	TN
Sodium lauryl sulfate	151-21-3	FP	FP
Ethylene glycol dimethacrylate	97-90-5	TP	TP
Xylene	1330-20-7	FN	FN
Sulfanilamide	63-74-1	TN	TN
2,4,6-Trinitrobenzenesulfonic acid	2508-19-2	FN	FN
2,3-Butanedione	431-03-8	TP	TP
2-Phenylpropionaldehyde	93-53-8	TP	TP
4-Allylanisole	140-67-0	TP	TP
Benzylidene acetone	122-57-6	TP	TP
Diethyl maleate	141-05-9	TP	TP
Fumaric acid	110-17-8	TN	TN
Glucose	50-99-7	TN	TN
Hydroxycitronellal	107-75-5	TP	TP
p-Aminobenzoic acid	150-13-0	TN	TN
Phthalic anhydride	85-44-9	FN	FN
Undecylenic acid	112-38-9	TP	TP
Vanillin	121-33-5	TN	TN
Propyl-4-hydroxybenzoate	94-13-3	FP	FP
Tartaric acid	87-69-4	TN	TN
n-Hexane	110-54-3	TN	TN
Hexadecyltrimethylammonium bromid	57-09-0	TN	TN
Glycerol	56-81-5	TN	TN
1,2-Propandiol	57-55-6	TN	TN

[1] Classifications revealed from Urbisch et al. (2015); Bauch et al. (2012).

Tab. S3.4: Results from applying different borderline ranges to the experimental set of substances (tested with the LLNA)[1]

Substance	CAS no.	*SDp* = 0.353 (BR = 2.65-3.53)	*SDp* = 0.498 (BR = 2.5-3.5)	*SDp* = 0.639 (BR = 2.36-3.64)	*SDp* = 0.709) (BR = 2.201-3.71
Salicylic acid	69-72-7	N	N	N	N
Methyl methacrylate	80-62-6	P	P	P	P
Chlorobenzene	108-90-7	N	N	N	N
Nickel chloride	7718-54-9	N	N	N	N
Phenyl benzoate	93-99-2	P	P	P	P
Methyl salicylate	119-36-8	N	N	N	N
MCI / MI	26172-55-4 & 2682-20-4	P	P	P	P
DL-Lactic acid	50-21-5	N	N	N	N
2-Mercaptobenzothiazole	149-30-4	P	P	P	P
Sodium dodecyl sulfate	151-21-3	P	P	P	P
Imidazolidinyl urea	39236-46-9	P	P	P	P
Ethylenglycolmethacrylate (EGDMA)	97-90-5	P	P	P	P
Cinnamic alcohol	104-54-1	P	P	P	P
Isopropanol	67-63-0	N	N	N	N
Isoeugenol	97-54-1	P	P	P	P
Xylene	1330-20-7	P	P	P	P
Alpha-Hexylcinnamaldehyde	101-86-0	P	P	P	P
p-Phenylenediamine	106-50-3	P	P	P	P
Citral	5392-40-5	P	P	P	P
Cobalt(II) chloride	7646-79-9	P	P	P	P
Eugenol	97-53-0	P	P	P	P
1-Chloro-2,4-dinitrobenzene (DNCB)	97-00-7	P	P	P	P

[1] Classifications revealed from Urbisch et al. (2015).

Tab. S3.5: Results from applying different borderline ranges to the experimental set of substances tested with the "2-out- of-3" ITS (results compared to the LLNA)[1]

Substance	CAS no.	LLNA
Phenyl benzoate	93-99-2	P
Ethylene diamine	107-15-3	P
Methyldibromo glutaronitrile (MDGN)	35691-65-7	P
Propyl gallate	121-79-9	P
Propylene glycol (1,2-Propanediol)	57-55-6	TN
Tartaric acid	87-69-4	TN
Glycerol	56-81-5	TN
n-Hexane	110-54-3	TN
Propyl paraben (Propyl-4-Hydroxybenzoate)	99-76-3	FP
Sulfanilamide	63-74-1	TN
Vanillin	121-33-5	TN
Isopropanol	67-63-0	TN
Lactic acid	50-21-5	TN
Methyl salicylate	119-36-8	TN
Fumaric acid	110-17-8	TN
Glucose	50-99-7	TN
p-Aminobenzoic acid	150-13-0	TN
Hexadecyltrimethylammonium bromide (Cetrimide)	57-09-0	TN
Xylene	1330-20-7	FN
Methylmethacrylate	80-62-6	TP
Aniline	62-53-3	FN
Ethylene glycol dimethacrylate (EGDMA)	97-90-5	TP
Undecylenic acid	112-38-9	TP
Hydroxycitronellal	107-75-5	TP
Cinnamyl Alcohol	104-54-1	TP
4-Allylanisole	140-67-0	TP
Sodium lauryl sulfate / sodium dodecyl sulfate (SDS)	151-21-3	FN
Farnesal	502-67-0	TP
2,3-Butanedione	431-03-8	TP
Citral	5392-40-5	TP
2-Phenylpropionaldehyde	93-53-8	TP
Benzylidene acetone (4-Phenyl-3-buten-2-one)	122-57-6	TP
Diethyl maleate	141-05-9	TP
Cobalt chloride	7646-79-9	TP
2,4,6-Trinitrobenzenesulfonic acid	2508-19-2	TP
Phthalic anhydride	85-44-9	FN
1,4-Phenylenediamine	106-50-3	TP
1-Chloro-2,4-dinitrobenzene (Dinitrochlorobenzene, DNCB)	97-00-7	TP
p-Benzoquinone	106-51-4	TP
MCI/MI	26172-55-4 & 2682-20-4	TP

[1] All possible combinations of the BR applied to individual methods revealed the same substances falling in the BR. Classifications revealed from Urbisch et al. (2015).