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Mechanism-Based QSAR Modeling of Skin Sensitization

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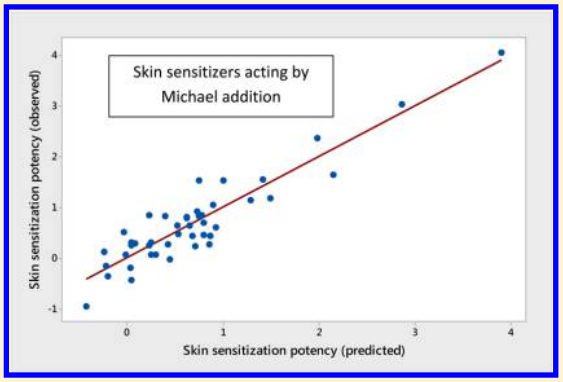
ABSTRACT: Many chemicals can induce skin sensitization, and there is a pressing need for non-animal methods to give a quantitative indication of potency. Using two large published data sets of skin sensitizers, we have allocated each sensitizing chemical to one of 10 mechanistic categories and then developed good QSAR models for the seven categories that have a sufficient number of chemicals to allow modeling. Both internal and external validation checks showed that each model had good predictivity.



INTRODUCTION

Skin sensitization (allergic contact dermatitis) is a common problem arising from the contact of certain chemicals with the skin. Once sensitized, an individual remains so for life, and it is therefore important to know whether a chemical possesses skin sensitization potential before skin contact is made.

In order for skin sensitization to be induced, a chemical must first penetrate into the viable epidermis and bind to skin proteins/peptides to form an immunogenic complex.[1] The binding is almost always covalent, with the chemical (hapten) acting as an electrophile and the protein as nucleophile; a few haptens operate via a free radical mechanism.[2] The immunogenic complex is taken up by dendritic cells, which convert the complex into a form that can be recognized by T-cells, causing their stimulation and proliferation and the formation of so-called memory T-cells; this is the induction process.[3] Upon re-exposure, the memory T-cells release cytotoxic mediators that cause local tissue inflammation.

A number of methods are available for the determination of skin sensitization potential; the current method of choice, and the one initially required for regulatory purposes,[4] is the LLNA,[5,6] which yields a quantitative end point. Much work has also been done on *in silico* prediction of skin sensitization potential in order to reduce animal usage and save time; this has become more important with the advent of the recent REACH legislation,[7,8] which requires assessment of toxicity for all chemicals produced in or imported into the European Union at levels above 1 tonne per annum but which also requires animal testing to be carried out only as a last resort.[9]

Despite the LLNA's having a quantitative end point, most *in silico* prediction studies of skin sensitization to date have been categorical (i.e., sensitizer/nonsensitizer),[10] as have most other attempts to use biological assays. A small number have used classical QSAR regression to model the LLNA end points of, for example, Schiff base electrophiles (aldehydes and ketones),[11] Michael acceptors,[12] S_NAr electrophiles,[13] and diverse organic chemicals.[14] Roberts and Patlewicz[15] have reviewed the subject.

In order to develop good QSAR models, all chemicals used in the training set should exert their effect by the same mechanism. Since it is often difficult to determine mechanisms of action, the default position has been to use chemicals of the same class (e.g., benzoic acids,[16] nitrobenzenes[17]) in the expectation that they have a common mechanism. However, with the emphasis in recent years on mechanistically based QSAR modeling and with current knowledge of mechanisms involved in skin sensitization,[18] we decided to try to use this approach to model the relatively large data sets of Gerberick et al.[19] and Kern et al.,[20] comprising 211 chemicals and 108 chemicals, respectively.

METHODS

Skin Sensitization Data. The Gerberick et al.[19] and Kern et al.[20] data sets contain a total of 85 nonsensitizers, which, of course, cannot be included in MLR modeling. In addition, two chemicals (cinnamic aldehyde and 2-amino-6-chloro-4-nitrophenol) were duplicated in the data sets. In the case of cinnamic aldehyde, for one duplicate there was some difference between the EC3 value of 1.4 reported by Gerberick et al.[19] and the value of 2.05 reported in the original publication;[21] in addition, the original publication[21] reported that the value of 2.05 was an average, indicating that a range of values had been obtained. Because of the doubt about the true EC3 value, we selected the other duplicate, with

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Table 1. Chemicals Used in This Study as Well as Their Potencies and Mechanisms of Action

name	CAS No.	MW	EC3	class	SSP	mechanism
4′-Hydroxychalcone	2657-25-2	224.26	0.002	Extreme	4.050	MA
p-Benzoquinone[a]	106-51-4	108.10	0.0099	Extreme	3.038	MA
2′,3′,4′-Trihydroxychalcone	1482-74-2	256.25	0.11	Strong	2.367	MA
Methyl 2-octynoate	111-12-6	154.21	0.45	Strong	1.535	MA
2′,4′-Dihydroxychalcone	1776-30-3	240.26	0.56	Strong	1.632	MA
Isopropyl isoeugenol	2953-00-7	206.29	0.6	Strong	1.536	MA
β-Phenylcinnamaldehyde	1210-39-5	208.26	0.6	Strong	1.540	MA
Isoeugenol[a]	97-54-1	164.20	1.2	Moderate	1.136	MA
2-Hydroxyethyl acrylate[a]	818-61-1	116.12	1.4	Moderate	0.919	MA
3-Methyl-4-phenyl-1,2,5-thiadiazole-1,1-dioxide (MPT)	3775-21-1	208.24	1.4	Moderate	1.172	MA
6-Methylisoeugenol	13041-12-8	178.23	1.6	Moderate	1.047	MA
Vinylpyridine	100-43-6	105.14	1.6	Moderate	0.818	MA
5,5-Dimethyl-3-methylene-dihydro-2(3*H*)-furanone	29043-97-8	126.16	1.8	Moderate	0.846	MA
trans-Anethol[a]	104-46-1	148.21	2.3	Moderate	0.809	MA
trans-2-Decenal	3913-71-1	154.25	2.5	Moderate	0.790	MA
Methyl 2-nonynoate	111-80-8	168.24	2.5	Moderate	0.828	MA
3,4-Dinitrophenol	577-71-9	184.10	2.6	Moderate	0.850	MA
Cinnamic aldehyde	104-55-2	132.16	3	Moderate	0.644	MA
2,4-Hexadienal	142-83-6	96.13	3.5	Moderate	0.439	MA
3-Methylisoeugenol[a]	186743-29-3	178.23	3.6	Moderate	0.695	MA
Benzylidene acetone (4-phenyl-3-buten-2-one)	122-57-6	146.19	3.7	Moderate	0.597	MA
2,4-Heptadienal[a]	5910-85-0	110.16	4	Moderate	0.440	MA
Tropolone	533-75-5	122.12	4.3	Moderate	0.453	MA
5-Methyl-2-phenyl-2-hexenal	21834-92-4	188.27	4.4	Moderate	0.631	MA
α-Methylcinnamaldehyde	101-39-3	146.19	4.5	Moderate	0.512	MA
trans-2-Hexenal	6728-26-3	98.15	5.5	Moderate	0.252	MA
Diethyl maleate	141-05-9	172.18	5.8	Moderate	0.473	MA
1,1,3-Trimethyl-2-formylcyclohexa-2,1-diene (safranal)	116-26-7	150.22	7.5	Moderate	0.302	MA
Perillaldehyde	2111-75-3	150.22	8.1	Moderate	0.268	MA
1-(*p*-Methoxyphenol)-1-penten-3-one[a]	104-27-8	190.24	9.3	Moderate	0.311	MA
Linalool aldehyde	Not known[b]	168.24	9.5	Moderate	0.248	MA
2-Ethylhexyl acrylate	103-11-7	184.28	10	Weak	0.265	MA
α-Amylcinnamaldehyde	122-40-7	202.30	11	Weak	0.265	MA
α-Butylcinnamaldehyde	7492-44-6	188.27	11	Weak	0.233	MA
Hexyl cinnamaldehyde	101-86-0	216.32	11	Weak	0.294	MA
Butyl acrylate	141-32-2	128.17	11	Weak	0.066	MA
R-Carvone[a]	6485-40-1	150.22	12.9	Weak	0.066	MA
Benzyl cinnamate	103-41-3	238.29	18.4	Weak	0.112	MA
Methyl acrylate[a]	96-33-3	86.09	20	Weak	−0.366	MA
Cinnamic alcohol	104-54-1	134.18	21	Weak	−0.195	MA
α-iso-Methylionone	127-51-5	206.33	21.8	Weak	−0.024	MA
Ethyl acrylate	140-88-5	100.12	28	Weak	−0.447	MA
Ethylene glycol dimethacrylate	97-90-5	198.22	28	Weak	−0.150	MA
2,2-*bis*-[4-(2-Hydroxy-3-methacryloxypropoxy)phenyl]-propane	1565-94-2	512.65	45	Weak	0.057	MA
Methyl methacrylate	80-62-6	100.12	90	Weak	−0.954	MA
Bandrowski's base	20048-27-5	318.38	0.04	Extreme	2.901	p-MA
3,4-Diaminonitrobenzene	99-56-9	153.14	0.05	Extreme	2.486	p-MA
4-((2-Hydroxyethyl)amino)-3-nitrophenol	65235-31-6	198.18	0.07	Extreme	2.452	p-MA
1,4-Dihydroquinone	123-31-9	110.11	0.11	Strong	2.000	p-MA
1,4-Phenylenediamine	106-50-3	108.14	0.16	Strong	1.830	p-MA
2,5-Diaminotoluene	95-70-5	122.08	0.2	Strong	1.786	p-MA
4-Amino-3-nitrophenol	610-81-1	154.12	0.2	Strong	1.887	p-MA
Lauryl gallate (dodecyl gallate)[a]	1166-52-5	338.44	0.3	Strong	2.052	p-MA
2-Aminophenol	95-55-6	109.13	0.4	Strong	1.436	p-MA
2-Methyl-5-hydroxyethylaminophenol	55302-96-0	167.21	0.4	Strong	1.621	p-MA
2-Nitro-*p*-phenylenediamine[a]	5307-14-2	153.14	0.4	Strong	1.583	p-MA
1,3-Phenylenediamine[a]	108-45-2	108.14	0.49	Strong	1.344	p-MA
R-Carvoxime	55658-55-4	165.23	0.6	Strong	1.440	p-MA
Hydroxytyrosol	10897-60-1	154.16	0.6	Strong	1.410	p-MA
1,2-Dibromo-2,4-dicyanobutane	35691-65-7	265.94	0.9	Strong	1.471	p-MA
1-Naphthol	90-15-3	144.17	1.3	Moderate	1.045	p-MA

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Table 1. continued

name	CAS No.	MW	EC3	class	SSP	mechanism
4-Amino-3-methylphenol	2835-99-6	123.15	1.45	Moderate	0.929	p-MA
2-(4-Amino-2-nitrophenylamino)-ethanol	2871-01-4	197.19	2.2	Moderate	0.952	p-MA
3-Aminophenol	591-27-5	109.13	3.2	Moderate	0.533	p-MA
5-Amino-2-methylphenol[a]	2835-95-2	123.15	3.4	Moderate	0.559	p-MA
3-Bromomethyl-5,5-dimethyl-dihydro-2(3*H*)-furanone	154750-20-6	207.07	3.6	Moderate	0.760	p-MA
2-Methoxy-4-methyl-phenol	93-51-6	138.17	5.8	Moderate	0.377	p-MA
Anisyl alcohol	105-13-5	138.17	5.9	Moderate	0.370	p-MA
Dihydroeugenol	2785-87-7	166.22	6.8	Moderate	0.388	p-MA
2-Amino-6-chloro-4-nitrophenol[a]	6358-09-4	188.57	6.85	Moderate	0.440	p-MA
1-Amino-2-nitro-4-bis(2-hydroxyethyl)-amino-benzene	29705-39-3	241.24	8.2	Moderate	0.469	p-MA
Eugenol	97-53-0	164.20	13	Weak	0.101	p-MA
5-Methyleugenol	186743-25-9	178.23	13	Weak	0.137	p-MA
6-Methyleugenol	186743-24-8	178.23	17	Weak	0.021	p-MA
4-Allylanisole	140-67-0	148.21	18	Weak	−0.084	p-MA
2,2′-Azobisphenol[a]	2050-14-8	214.20	27.9	Weak	−0.115	p-MA
3-Methyleugenol	186743-26-0	178.23	32	Weak	−0.254	p-MA
Glutaraldehyde	111-30-8	100.12	0.1	Strong	2.001	SB
Chloroatranol	57074-21-2	186.59	0.4	Strong	1.669	SB
Atranol[a]	526-37-4	152.15	0.6	Strong	1.404	SB
Formaldehyde	50-00-0	30.03	0.61	Strong	0.692	SB
1-Phenyl-1,2-propanedione	579-07-7	148.16	1.3	Moderate	1.057	SB
Glyoxal	107-22-2	58.04	1.4	Moderate	0.618	SB
Methyl pyruvate[a]	600-22-6	102.09	2.4	Moderate	0.629	SB
Phenylacetaldehyde	122-78-1	120.15	3.0	Moderate	0.603	SB
α-Methylphenylacetaldehyde	93-53-8	134.18	6.3	Moderate	0.328	SB
Undec-10-enal	112-45-8	168.28	6.8	Moderate	0.394	SB
1-(2′,3′,4′,5′-Tetramethylphenyl)butane-1,3-dione	167998-73-4	218.30	8.3	Moderate	0.420	SB
1-(2′,5′-Diethylphenyl)butane-1,3-dione	167998-76-7	218.30	9.6	Moderate	0.357	SB
Camphorquinone	465-29-2	166.22	10	Weak	0.221	SB
2-Methylundecanal	110-41-8	184.32	10	Weak	0.266	SB
2,3-Butanedione[a]	431-03-8	86.09	11	Weak	−0.106	SB
1-Phenyloctane-1,3-dione	55846-68-1	218.30	11	Weak	0.298	SB
Farnesal	502-67-0	220.36	12	Weak	0.264	SB
Citral	5392-40-5	152.44	13	Weak	0.069	SB
1-(2′,5′-Dimethylphenyl)butane-1,3-dione	56290-55-2	190.24	13	Weak	0.165	SB
4-Methylhydrocinnamic aldehyde	5406-12-2	148.21	14	Weak	0.025	SB
α-Methyl-1,3-benzodioxole-5-propionaldehyde[a]	1205-17-0	192.21	16.4	Weak	0.069	SB
3- and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde	31906-04-4	210.32	17	Weak	0.092	SB
4-*tert*-Butyl-α-ethylhydrocinnamal	80-54-6	204.31	19	Weak	0.032	SB
N,*N*-Dibutylaniline[ac]	613-29-6	205.30	19.6	Weak	0.020	SB
4,4,4-Trifluoro-1-phenylbutane-1,3-dione	326-06-7	216.16	20	Weak	0.034	SB
4,4′-Dibromobenzil[ac]	35578-47-3	368.02	20.5	Weak	0.254	SB
Cyclamen aldehyde[ad]	103-95-7	190.29	22	Weak	−0.063	SB
cis-6-Nonenal	2277-19-2	140.23	23	Weak	−0.215	SB
5-Methyl-2,3-hexanedione	13706-86-0	128.17	26	Weak	−0.307	SB
2,2,6,6-Tetramethyl-heptane-3,5-dione	1118-71-4	184.28	27	Weak	−0.166	SB
1-Phenyl-2-methylbutane-1,3-dione	6668-24-2	176.22	29	Weak	−0.216	SB
3-Ethoxy-1-(2′,3′,4′,5′-tetramethylphenyl)propane-1,3-dione	170928-69-5	248.32	33	Weak	−0.124	SB
Hydroxycitronellal	107-75-5	172.27	33	Weak	−0.282	SB
2-(4-*tert*-Amylcyclohexyl)acetaldehyde[a]	620159-84-4	196.33	37	Weak	−0.275	SB
Diethyl acetaldehyde	97-96-1	100.16	76	Weak	−0.880	SB
3-(Dimethylamino)propylamine	109-55-7	102.18	2.2	Moderate	0.667	p-SB
Ethylenediamine	107-15-3	60.10	2.2	Moderate	0.436	p-SB
Diethylenetriamine[ad]	111-40-0	103.17	5.8	Moderate	0.250	p-SB
3-Methyl-1-phenylpyrazolone	89-25-8	174.20	8.5	Moderate	0.312	p-SB
Geraniol	106-24-1	154.25	26	Weak	−0.227	p-SB
1-Chloromethylpyrene	1086-00-6	250.73	0.005	Extreme	3.700	S_N2
5-Chloro 2-methyl-4-isothiazolin-3-one	26172-55-4	149.60	0.009	Extreme	3.221	S_N2
1-Methyl-3-nitro-1-nitrosoguanidine	70-25-7	147.09	0.03	Extreme	2.690	S_N2
N-Methyl-*N*-nitrosourea	684-93-5	103.08	0.05	Extreme	2.314	S_N2
4-Nitrobenzyl bromide[a]	100-11-8	216.03	0.05	Extreme	2.636	S_N2

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Table 1. continued

name	CAS No.	MW	EC3	class	SSP	mechanism
β-Propiolactone	57-57-8	72.06	0.15	Strong	1.682	S_N2
Dimethyl sulfate[a]	77-78-1	126.13	0.19	Strong	1.822	S_N2
Benzyl bromide	100-39-0	171.04	0.2	Strong	1.932	S_N2
Methyl dodecanesulfonate	2374-65-4	264.42	0.39	Strong	1.831	S_N2
Iodopropynyl butylcarbamate	55406-53-6	281.09	0.9	Strong	1.495	S_N2
N-Ethyl-*N*-nitrosourea	759-73-9	117.11	1.1	Moderate	1.027	S_N2
Bisphenol A-diglycidyl ether	1675-54-3	340.42	1.5	Moderate	1.356	S_N2
2-Methyl-2*H*-isothiazol-3-one[a]	2682-20-4	115.15	1.9	Moderate	0.783	S_N2
1,2-Benzisothiazolin-3-one	2634-33-5	151.18	2.3	Moderate	0.818	S_N2
1-Bromohexadecane	112-82-3	305.34	2.3	Moderate	1.123	S_N2
Benzyl salicylate	118-58-1	228.25	2.9	Moderate	0.896	S_N2
Diethyl sulfate	64-67-5	154.18	3.3	Moderate	0.670	S_N2
2-Bromotetradecanoic acid[a]	10520-81-7	307.27	3.4	Moderate	0.956	S_N2
1-Bromoheptadecane	3508-00-7	319.37	4.8	Moderate	0.823	S_N2
1-Bromopentadecane	629-72-1	291.32	5.1	Moderate	0.757	S_N2
Tetramethylthiuram disulfide	137-26-8	240.42	5.2	Moderate	0.665	S_N2
1-Bromoeicosane	4276-49-7	361.45	6.1	Moderate	0.773	S_N2
2-Bromoethylbenzene	103-63-9	185.10	6.2	Moderate	0.475	S_N2
12-Bromo-1-dodecanol[a]	3344-77-2	265.24	6.9	Moderate	0.585	S_N2
Methyl methanesulfonate	66-27-3	110.13	8.1	Moderate	0.133	S_N2
1-Bromodocosane	6938-66-5	389.51	8.3	Moderate	0.671	S_N2
Dodecyl methanesulfonate	51323-71-8	264.42	8.8	Moderate	0.478	S_N2
1-Chlorohexadecane	4860-03-1	260.89	9.1	Moderate	0.457	S_N2
1-Bromotetradecane	112-71-0	277.29	9.2	Moderate	0.479	S_N2
1-Bromohexane	111-25-1	165.07	10	Weak	0.218	S_N2
1-Bromotridecane	765-09-3	263.26	10	Weak	0.420	S_N2
1-Iodododecane	4292-19-7	296.24	13	Weak	0.358	S_N2
1-Iodotetradecane[a]	19218-94-1	324.29	14	Weak	0.365	S_N2
1-Bromooctadecane[a]	112-89-0	333.40	15	Weak	0.347	S_N2
1-Chlorooctadecane	3386-33-2	288.95	16	Weak	0.257	S_N2
Benzyl benzoate	120-51-4	212.25	17	Weak	0.096	S_N2
1-Bromododecane[a]	143-15-7	249.24	18	Weak	0.141	S_N2
12-Bromododecanoic acid	73367-80-3	279.22	18	Weak	0.191	S_N2
1-Iodohexadecane	544-77-4	352.35	19	Weak	0.268	S_N2
1-Bromoundecane	693-67-4	235.21	20	Weak	0.070	S_N2
1-Chlorotetradecane	2425-54-9	232.84	20	Weak	0.066	S_N2
7-Bromotetradecane	74036-97-8	277.29	21	Weak	0.121	S_N2
1-Iodononane[a]	4282-42-2	254.16	24	Weak	0.025	S_N2
Oleyl methanesulfonate	35709-09-2	346.57	25	Weak	0.142	S_N2
Butyl glycidyl ether	2426-08-6	130.19	31	Weak	−0.377	S_N2
Benzo[*a*]pyrene	50-32-8	252.32	0.0009	Extreme	4.448	p-S_N2
7,12-Dimethylbenz[*α*]anthracene	57-97-6	256.35	0.006	Extreme	3.631	p-S_N2
4-Ethoxymethylene-2-phenyl-2-oxazolin-5-one	15646-46-5	217.22	0.003	Extreme	3.860	Ac
Tetrachlorosalicylanilide[a]	1154-59-2	351.02	0.04	Extreme	2.943	Ac
Fluorescein-5-isothiocyanate	3326-32-7	389.38	0.14	Strong	2.444	Ac
2-Methyl -4*H*,3,1-benzoxazin-4-one	525-76-8	161.16	0.7	Strong	1.362	Ac
C6 Azlactone	176665-02-4	197.28	1.3	Moderate	1.181	Ac
2-Mercaptobenzothiazole	149-30-4	167.24	1.7	Moderate	0.993	Ac
C4 Azlactone	176664-99-6	169.22	1.8	Moderate	0.973	Ac
Nonanoyl chloride	764-85-2	176.69	1.8	Moderate	0.992	Ac
Methyl 2-sulfophenyl octadecanoate	Not known[b]	454.67	2	Moderate	1.357	Ac
Isononanoyl chloride[a]	57077-36-8	176.69	2.7	Moderate	0.816	Ac
3,5,5-Trimethylhexanoyl chloride	36727-29-4	176.69	2.7	Moderate	0.816	Ac
C9 Azlactone	176665-04-6	239.36	2.8	Moderate	0.932	Ac
3-Propylidenephthalide	17369-59-4	174.20	3.7	Moderate	0.673	Ac
3,4-Dihydrocoumarin	119-84-6	148.16	5.6	Moderate	0.423	Ac
Palmitoyl chloride[a]	112-67-4	274.88	8.8	Moderate	0.495	Ac
1,2,4-Benzenetricarboxylic anhydride	552-30-7	192.13	9.2	Moderate	0.320	Ac
C11 Azlactone	176665-06-8	267.41	16	Weak	0.223	Ac
C15 Azlactone	176665-09-1	323.52	18	Weak	0.255	Ac
C17 Azlactone	176665-11-5	351.58	19	Weak	0.267	Ac

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Table 1. continued

name	CAS No.	MW	EC3	class	SSP	mechanism
Phenyl benzoate	93-99-2	198.22	20	Weak	−0.004	Ac
Imidazolidinylurea	39236-46-9	388.30	24	Weak	0.209	Ac
C19 Azlactone[a]	Not known[b]	379.63	26	Weak	0.164	Ac
Penicillin G	61-33-6	334.39	30	Weak	0.047	Ac
5-Chlorosalicylanilide	4638-48-6	247.68	5	Moderate	0.695	OxPot
α-Phellandrene	99-83-2	136.23	5.4	Moderate	0.402	OxPot
β-Phellandrene[a]	555-10-2	136.23	5.6	Moderate	0.386	OxPot
(5*R*)-5-Isopropenyl-2-methyl-1-methylene-2-cyclohexene	Not known[b]	148.25	7.3	Moderate	0.308	OxPot
2-(Hexadecyloxy)ethanol	2136-71-2	286.50	8.8	Moderate	0.513	OxPot
α-Terpinene	99-86-5	136.24	8.9	Moderate	0.185	OxPot
Acetyl cedrene	32388-55-9	246.39	13.9	Weak	0.249	OxPot
Abietic acid	514-10-3	302.46	15	Weak	0.305	OxPot
Linalool	78-70-6	154.25	30	Weak	−0.289	OxPot
R(+) Limonene	5989-27-5	136.24	69	Weak	−0.705	OxPot
Aniline[a]	62-53-3	93.13	89	Weak	−0.980	OxPot
Chlorothalonil	1897-45-6	265.91	0.004	Extreme	3.823	S_NAr
1-Chloro-2,4-dinitrobenzene	97-00-7	202.55	0.05	Extreme	2.608	S_NAr
2,4,6-Trichloro-1,3,5-triazine	108-77-0	184.41	0.09	Extreme	2.312	S_NAr
Pentachlorophenol	87-86-5	266.34	20	Weak	0.124	S_NAr
Clotrimazole	23593-75-1	344.85	4.8	Moderate	0.856	S_N1
D,L-Citronellol	106-22-9	156.27	43.5	Weak	−0.445	S_N1

[a]These chemicals were used as test set chemicals. Those marked [ac] were used only in the SB test set, and those marked [ad] were used only in the SB + p-SB test set. [b]For compounds with unknown CAS numbers, the SMILES strings are linalool aldehyde, C=CC(C)(O)CCC=C(C)C=O; methyl 2-sulfophenyl octadecanoate, CCCCCCCCCCCCCCCCC(C)C(=O)Oc1ccccc1S(O)(=O)=O; C19 azlactone, CCCCCCCCCCCCCCC-CCCCC1=NC(C)(C)C(=O)O1; and (5*R*)-5-isopropenyl-2-methyl-1-methylene-2-cyclohexene, CC(=C)[C@@H]1CC=C(C)C(=C)C1

an EC3 value of 3.0. In the case of 2-amino-6-chloro-4-nitrophenol, we rejected one EC3 value (2.2), as it was obtained from an erratic dose−response curve. One chemical (*bis*-3,4-epoxycyclohexyl-ethyl-phenyl-methylsilane) contained silicon, and several were ionic chemicals, which could not be handled by our software. Isopropyl myristate was removed because it was listed as a false positive,[19] and methyl hexadecene sulfonate was deleted because the molecular structure and CAS numbers given in Gerberick et al.[19] are incorrect. These deletions left a total of 204 skin sensitizers for modeling.

The LLNA involves the topical exposure of the ear dorsum of CBA female mice to 25 μL of at least three different concentrations of test chemical daily for 3 days. After a further 2 days, an injection is given of 250 μL of phosphate-buffered saline containing 20 μCi of tritiated thymidine. Five hours later, the animals are sacrificed, the draining auricular lymph nodes are excised, and the incorporation of tritiated thymidine is measured. From these results, the EC3 value is calculated.

It should be noted that EC3 values are reported as g/100 mL. Four potency ranges are used, as follows: EC3 ≥ 10 to ≤ 100, weak; EC3 ≥ 1 to < 10, moderate; EC3 ≥ 0.1 to < 1, strong; and EC3 < 0.1, extreme.[19] Use of weight concentrations can give rise to a classification problem. Strictly, concentrations and dosages should be given in molar units (e.g., mmol L^{-1}, μmol kg^{-1}) for comparison because effects are initiated by the number of molecules present, not by how much they weigh.[22] Hence, we have used SSP, defined as SSP = log(MW/10EC3), in our modeling. The importance of this is demonstrated by two chemicals from our data set, formaldehyde (MW 30.03) and 3-methylisoeugenol (MW 178.23). They have almost identical skin sensitization potencies (1.692 and 1.695) based on their molar concentrations, yet their EC3 values are quite different (0.61 and 3.6%), meaning that formaldehyde is classified as a strong sensitizer, whereas 3-methylisoeugenol is classified as a moderate sensitizer.

Using our in-house expertise,[18] now incorporated into the Toxtree software,[23] together with additional expert knowledge (D.W.R. and S.J.E.), we classified the chemicals into their mechanistic categories. The chemicals are listed in Table 1. We have retained the chemical names used by Gerberick et al.[19] and Kern et al.[20] for ease of cross-reference and have included CAS numbers for all of the 204 chemicals, save for four chemicals whose CAS numbers we were unable to find.

QSAR Modeling. It is widely acknowledged that for a QSAR model to be predictive external test chemicals should be similar to one or more chemicals in the training set used to build the model.[24−26] There are a number of methods used to achieve this,[27] although the topic is still open and has not been completely solved.[28] Perhaps the most widely practiced approach is that using a clustering technique on the whole data set in order to select test set chemicals that are similar to one or more chemicals in the remaining chemicals (i.e., the training set).

It has also been pointed out[24,29] that external test set chemicals should, strictly speaking, be completely independent of the training set. However, the clustering technique does not comply with that requirement,[22,29] since the selection of test chemicals that are very similar to chemicals in the training set means that they carry the same structural information.[30]

In addition, for relatively small data sets such as ours, removal of even a small number of test set chemicals results in loss of a significant amount of information.[31] This is of even more concern when the data set comprises chemicals of a range of chemical classes, as is the case with our skin sensitizers (see Table 1). It is thus likely that the use of leave-many-out and bootstrap techniques[24] would also be inappropriate.

Using the clustering technique for selection of test chemicals, Gramatica et al.[32] found that the four descriptors used to develop a good 93-chemical training set QSAR for K_{oc} prediction (R^2 = 0.82, SE = 0.539) also yielded a good QSAR on the whole 643-chemical data set (R^2 = 0.79, SE = 0.547). However, this was not the case with our small data sets. For example, for the Michael acceptor chemicals, a 6-descriptor QSAR developed using the 36-chemical training set had R^2 = 0.866, SE = 0.344. When the same 6 descriptors were used to develop a QSAR for all 45 Michael acceptor chemicals, the result was poor (R^2 = 0.636, SE = 0.570). This confirms the view of Roy et al.[31] that removal of test set chemicals from a small data set results in loss of information and thus changes the applicability domain of the model. Partly for this reason, Hawkins[33] recommended that external validation should not be carried out on data sets much fewer 50 chemicals, whereas Tropsha[27] recommended a minimum of 30−40 chemicals and Gramatica[34] recommended a minimum of 25 chemicals. From Table 1, it can be seen that our data sets range in size from 11 to 45 chemicals and thus are at least verging on the size where external validation may be expected not

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Chem. Res. Toxicol. 2015, 28, 1975−1986

Table 2. Models Developed in This Work for Skin Sensitization

mech.	model	eq no.	no. of chemicals		equation		R^2 (R^2_{adj})	Q^2	SE	F	p values
All	Full	1	204	SSP =	−1.164(0.282) + 1.759(0.450) FASA- + 0.174(0.028) eaC2C3a + 0.807(0.155) vsurf_CW2 + 0.012(0.0026) vsurf_D8 − 0.767 (0.202) Hmin − 0.190(0.057) SHCsatu		0.496 (0.480)	0.459	0.689	32.4	<0.001
MA	Full	2	45	SSP =	16.7(2.52) − 0.101(0.020) S4 − 0.760(0.174) HS17 + 0.112(0.015) SlogP_VSA4 + 0.775(0.195) vsurf_CW2 − 8.39(1.14) Max. BC1 − 43.4(7.37) Rel. PMI		0.856 (0.834)	0.793	0.358	37.8	<0.001
MA	Train	3	36	SSP =	16.6(3.77) − 0.094(0.029) S4 − 0.743(0.201) HS17 + 0.113(0.017) SlogP_VSA4 + 0.673(0.257) vsurf_CW2 − 8.26(1.78) Max. BC1 − 42.2(9.9) Rel. PMI		0.825 (0.789)	0.692	0.398	22.9	≤0.015
MA	Test	4	9	SSP (obsd) =	−0.113 + 1.12 SSP (pred)	(ICC = 0.977)	0.965	0.937	0.191	195.9	
p-MA	Full	5	32	SSP =	−0.360(0.369) + 1.400(0.194) S24 − 0.319(0.046) e1C3O2a + 0.279(0.085) SssNH − 0.337(0.051) vsurf_HB7 + 0.467(0.108) Av. IC2		0.858 (0.831)	0.790	0.349	31.4	≤0.003
p-MA	Train	6	26	SSP =	−0.139(0.454) + 1.348(0.249) S24 + 0.254(0.097) SssNH − 0.318(0.057) e1C3O2a − 0.359(0.098) vsurf_HB7 + 0.401(0.131) Av. IC2		0.848 (0.810)	0.768	0.380	22.3	≤0.01
p-MA	Test	7	6	SSP (obsd) =	0.039 + 0.958 SSP (pred)	(ICC = 0.951)	0.887	0.758	0.305	31.5	
SB	Full	8	35	SSP =	−6.99(1.47) + 0.090(0.020) S7 + 0.035(0.014) S10 − 3.107(0.717) GCUT_PEOE_1 + 1.880(0.496) vsurf_Wp7 + 2.657(0.702) Av. SI2 + 3.101(1.084) Av. BO + 0.177(0.026) Kier FI		0.837 (0.795)	0.644	0.259	19.9	≤0.02
SB	Train	9	28	SSP =	−7.54(1.75) + 0.0853(0.0236) S7 + 0.042(0.016) S10 − 2.704(0.869) GCUT_PEOE_1 + 1.294(0.852) vsurf_Wp7 + 2.798(0.829) Av. SI2 + 3.573(1.250) Av. BO + 0.193(0.031) Kier FI		0.838 (0.781)	0.524	0.272	14.8	≤0.15
SB	Test	10	7	SSP (obsd) =	0.060 + 1.02 SSP (pred)		0.904	0.857	0.194	47.0	
SB + p-SB	Full	11	40	SSP =	19.22(2.95) + 0.380(0.086) HS6 − 0.238(0.058) dx2 − 0.0813(0.0107) E_sol + 0.0958(0.0173) Kier FI − 0.00153(0.00047) DPSA1 − 4.542(0.670) Av. valency − 5.885(1.066) relative no. O atoms − 5.885(1.066) relative no. O atoms		0.850 (0.817)	0.781	0.233	25.9	≤0.005
SB + p-SB	Train	12	33	SSP =	19.09(3.36) + 0.344(0.107) HS6 − 0.226(0.069) dx2 − 0.070(0.016) E_sol + 0.103(0.021) Kier FI − 0.00163(0.00053) DPSA1 − 4.490(0.760) Av. valency − 5.960(1.230) relative no. O atoms		0.836 (0.790)	0.736	0.251	18.2	≤0.005
SB + p-SB	Test	13	7	SSP (obsd) =	−0.143 + 1.27 SSP (pred)	(ICC = 0.936)	0.935	0.838	0.162	71.4	
S_N2	Full	14	45	SSP =	−9.468(1.304) + 0.109(0.034) S14 + 0.151(0.050) $SsCH_3$ + 4.004(0.717) xvp9 + 0.150(0.037) eaC2C3a + 8.780(0.864) FASA- + 3.496(0.589) PEOE_VSA_FPOS − 0.473(0.094) MNDO_HOMO		0.852 (0.823)	0.796	0.381	30.3	≤0.005
S_N2	Train	15	36	SSP =	−9.689 + 0.109(0.039) S14 + 0.149(0.058) $SsCH_3$ + 4.233(0.854) xvp9 + 0.142(0.042) eaC2C3a + 9.084(1.155) FASA- + 3.699(0.694) PEOE_VSA_FPOS − 0.477(0.123) MNDO_HOMO		0.837 (0.797)	0.773	0.419	20.6	≤0.02
S_N2	Test	16	9	SSP (obsd) =	−0.023 + 0.889 SSP (pred)		0.951	0.927	0.204	134.7	<0.001
Ac	Full	17	22	SSP =	0.873(0.088) − 0.616 (0.152) HS14 + 2.644(0.225) HS16 − 3.059(0.289) HS17 + 0.633 (0.122) HS29		0.921	0.886	0.304	49.5	<0.001
Ac	Train	18	18	SSP =	0.879(0.110) − 0.578(0.210) HS14 + 2.645(0.262) HS16 − 3.079(0.371) HS17 + 0.629(0.142) HS29 − 3.079(0.371) HS17 + 0.629(0.142) HS29		0.899 (0.867)	0.863	0.342	28.8	≤0.015
Ac	Test	19	4	SSP (obsd) =	−0.079 + 0.966 SSP (pred)	(ICC = 0.995)		0.999	0.992	0.042 2672.7	
OxPot	Full	20	11	SSP =	0.365(0.072) − 0.179(0.017) vsurf_DD12 + 0.0957(0.0200) vsurf_DD23		0.930 (0.912)	0.856	0.156	52.8	<0.001
OxPot	Train	21	9	SSP =	0.363(0.066) − 0.156(0.017) vsurf_DD12 + 0.081(0.018) vsurf_DD23		0.931 (0.908)	0.865	0.130	40.4	<0.001
OxPot	Test		2		No QSAR with only 2 test chemicals	(ICC = 0.945)					

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to perform well. It may be noted also that because of the diversity of our data sets, a greater number of descriptors is required to give good models.[26]

The above paragraph indicates that, because of the smallness and chemical diversity of our data sets, we could not expect to obtain good predictive models based on descriptors selected during development of the training sets. We therefore decided to use for the training sets the descriptors selected for the corresponding QSARs developed for the full data sets. We recognize that this means that the training set QSARs are not fully independent of the test set chemicals, but we believe that this is no less valid than the widely used clustering approach for the selection of test set chemicals, which also involves some loss of independence of test set chemicals. Our approach also means that the applicability domains of the full data sets are preserved to some extent at least and thus overcomes the concerns of Hawkins[33] and Gramatica[34] in that respect. We stress, however, that this approach should be used only for small, very diverse data sets, but in such cases, we believe that it fits with the dictum of Albert Einstein: *Everything should be made as simple as possible, but not simpler*.

There were too few chemicals acting by S_N1, pro-S_N2, and S_NAr mechanisms (2, 2, and 4 chemicals, respectively) to allow us to develop QSARs in these categories. Hence, 196 chemicals constituted our pool of chemicals used for modeling.

Various methods can be employed for the splitting of a data set into training and test sets, from random selection to activity sampling, clustering techniques, self-organizing maps, and formal statistical experimental design.[24] Random selection is intuitively unappealing and "could result in a subsequent application of the model out of its applicability domain, resulting in erroneous conclusions on the model's performance".[34] In addition, it does not provide any rationale for selection.[35] However, it was found to yield similar predictive power to methods based on clustering.[35] Activity sampling (e.g., ordering the chemicals according to their activity and then taking every *n*th chemical for the test set) ensures a good coverage of activity, but it does not necessarily take account of chemical diversity and thus again risks subsequent application outside the applicability domain. The other techniques can be complex[27] and can give conflicting results.[35] Tropsha et al.[24] have stated that "the underlying goal...is to ensure that both the training and test sets separately span the whole descriptor space occupied by the entire data set and the chemical domains in the two sets are not too dissimilar". Chirico and Gramatica[28] have commented that "the topic (of external validation) is still open, and the problem in QSAR modelling has not yet been completely solved, though many techniques have been proposed to validate models". The above approaches have been designed for large or relatively large data sets, and we did not have that luxury. In fact, the external validation of small heterogeneous data sets has not been addressed before. Martin et al.[35] have pointed out that rational design of test sets should ensure that "the compounds in the training and test sets should be close to each other". However, as stated earlier, selection of test chemicals that are very similar to chemicals in a training set means that they carry the same structural information,[30] which would lead to overestimation of the predictivity of the model. We therefore used a manual sampling approach that ensured a good range of activities and chemical domains in the test sets while never selecting the chemicals with the highest and lowest activities in the whole data sets[36] to avoid the risk of extrapolation of the training set models. Care was taken that the test set chemicals covered approximately the same chemical and biological space as the training set chemicals in each category and were not too close to or too far from the line of best fit in the relevant whole data set model.

It is likely that with small, heterogeneous data sets there is no entirely satisfactory way to demonstrate true prediction capability using QSAR modeling. We believe that the simple method that we have adopted, while not perfect, is acceptable and that the alternatives are open to at least as much criticism as the one that we have used. We recognize that our approach could be controversial, but we believe that it is a useful and pragmatic method for QSAR prediction using small, diverse data sets. We do not recommend it for use with large and/or homogeneous data sets. A reviewer has commented that the Q^2 (leave-one-out) value of each training set could be more valuable than the test set values. In fact, as can be seen from Table 2, all of our training set Q^2 values are above the recommended lower limit of 0.5[37] and are no more than the recommended[38] 0.3 below the corresponding R^2 value, with the exception of the Schiff base model, instead of which we recommend the combined Schiff base and pro-Schiff base model, which has good statistics (R^2 = 0.836, Q^2 = 0.736).

A total of about 1600 descriptors were generated from CODESSA,[39] MOE,[40] and winMolconn[41] software. These were pruned, by removal of descriptors with the same values for all chemicals and by removal of descriptors with high pairwise collinearity, to about 880 descriptors. Statistical analysis was carried out using the simple wrapper method of stepwise MLR[42] in Minitab v17 software[43] on the chemicals in each mechanistic category. Modeling was first performed on the total number of chemicals in each category. Then, approximately 20% of the chemicals in each category were removed to serve as a test set, and each model was redeveloped on the remaining (training set) chemicals, using the same descriptors as were obtained for the model developed with the total number of chemicals in the category. The predicted skin sensitization potencies of test set chemicals were calculated from the QSARs developed for the corresponding training set chemicals.

The number in brackets after each coefficient in a QSAR is the standard error on the coefficient. For a descriptor to be valid, the standard error on its coefficient should be significantly lower than the value of the coefficient itself. This is also reflected in the *p* value for each descriptor, a measure of the probability that the descriptor is there by chance; for a descriptor to be valid in a QSAR, its *p* value should generally be <0.05 (that is, less than a 5% risk that it is present by chance).

The statistics given with each QSAR are R^2 (indicating the proportion of the variation of skin sensitization potency (SSP) modeled by the QSAR); R^2_{adj} (which allows comparison between QSARs with different numbers of descriptors); Q^2 (an internal measure of predictivity, obtained using the leave-one-out procedure in Minitab); SE; and *F* (the Fisher statistic, an indication of the fit of the regression equation to the training set data).

We also carried out 20 Y-randomizations of the SSP values within each mechanism in order to check the robustness of the QSARs generated. For each mechanism, all R^2 values obtained using randomized SSP values were significantly lower than the values obtained with nonrandomized SSP values.

For the test set results, the correlation between observed and predicted SSP values should have an intercept close to zero and a slope close to unity. However, it has been pointed out that correlation alone is not an adequate criterion for agreement between predicted and observed values of biological end points.[24] To establish agreement, it is necessary to exclude three potential problems: (i) random disagreement, (ii) biased disagreement with one set of values being systematically greater than (or less than) the other, and (iii) gradient problems (the points on a graph of predicted versus observed values adhering to a line with a gradient other than +1.0). Tropsha et al.[24] have recommended a multistep procedure for assessing how well those criteria are met.

However, there is a simpler alternative, the ICC, that serves just as well and has been available for many years.[44] There are various ways in which the ICC can be calculated, but in some of its forms, it will produce a value close to +1.0 only if the data adhere tightly to all three of the criteria set out above. It can therefore act as a single unified indicator of agreement between predicted and observed values. In the event that the ICC value was low, the exact nature of the problem could be diagnosed by plotting the discrepancies between the values against the average of the two (Bland–Altman plot), as advised by Machin, Campbell, and Walters.[45] We have used the ICC to assess how well our test set data meet the above criteria. Weir[46] has pointed out that the ICC is conceptually akin to R^2 from regression, so it is reasonable to assume that a value that is considered good for R^2 (say, 0.9) can also be considered good for the ICC.

ICC values were calculated using the Reliability Analysis procedure in SPSS, v20.[47] The statistical model was set to Two-Way Mixed, and the ICC type was set to Absolute Agreement. The ICC values reported are for those for Single Measures.

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Table 3. Descriptors and SSPs Used in the QSAR Models and Their Ranges

All 204 Active Sensitizers
SSP (−0.980 to 4.050)
FASA-: MOE; fractional accessible surface area of all atoms with negative partial charge (0.067 to 0.703)
eaC2C3a: winMolconn; bond-type electrotopological state index for single bond between unsubstituted carbon and carbon with three aromatic neighbors (0 to 18.723)
vsurf_CW2: MOE; capacity factor (shape, volume, surface area descriptor) (1.160 to 3.211)
vsurf_D8: MOE; hydrophobic volume (0 to 112.88)
Hmin: CODESSA; minimum number of hydrogen bond donors and acceptors (0 to 1.514)
SHCsatu: winMolconn: number of hydrogen atoms on sp^3 carbons bonded to sp^2 carbons (0 to 4.407)
Michael Addition
SSP (−0.954 to 4.050)
S4: winMolconn; atom level E-State for atom 4 (−3.617 to 10.190)
HS17: winMolconn; hydrogen atom level HE-state for hydrogen atom 17 (0 to 2.690)
SlogP_VSA4: MOE; sum of van der Waals surface areas such that contribution to log P is in the range 0.1−0.15 (0 to 30.233)
vsurf_CW2: MOE; capacity factor (shape, volume, surface area descriptor) (1.352 to 2.836)
Max. BC1: CODESSA; maximum bonding contribution of one (1.84 to 2.14)
Rel. PMI: CODESSA; relative principal moment of inertia (0 to 0.05)
Pro-Michael Addition
SSP (−0.115 to 2.901)
S24: winMolconn; atom level E-state index for atom 24 (0 to 1.817)
e1C3O2a: winMolconn; bond-type E-state for single bond between ether oxygen and substituted aromatic carbon (0 to 3.311)
SssNH: winMolconn; atom type E-state index for >NH nitrogen (0 to 2.952)
vsurf_HB7: MOE; H-bond donor capacity (−3.125 to 3.375)
Av. IC2: CODESSA; average information content (_2), a structural descriptor (1.02 to 2.19)
Schiff Base
SSP (−0.880 to 2.001)
S7: winMolconn; atom level E-state for atom 7 (−0.526 to 11.481)
S10: winMolconn; atom level E-state for atom 10 (−2.017 to 10.595)
GCUT_PEOE_1: MOE; the GCUT descriptors are calculated from the eigenvalues of a modified graph distance adjacency matrix. Each *ij* entry of the adjacency matrix takes the value 1/sqr(d_{ij}), where d_{ij} is the (modified) graph distance between atoms *i* and *j*. The diagonal takes the value of the PEOE partial charges. The resulting eigenvalues are sorted, and the smallest, 1/3-ile, 2/3-ile, and largest eigenvalues are reported (−0.468 to −0.187)
vsurf_Wp7: MOE; polar volume (shape, volume, surface area descriptors) (0 to 0.50)
Av. SI2: CODESSA; average structural information_2, a structural descriptor (0.35 to 0.92)
Schiff Base
Av. BO: CODESSA; average bond order (0.96 to 1.13)
Kier FI: CODESSA; Kier flexibility index (1.25 to 13.94)
Schiff Base + Pro-Schiff Base
SSP (−0.880 to 2.001)
HS6: winMolconn; hydrogen atom level HE-state for hydrogen atom 6 (0 to 1.391)
dx2: winMolconn; second-order connectivity index difference between a molecule and its unbranched isomer (0 to 2.588)
E_sol: MOE; solvation energy (−20.623 to −4.438)
Kier FI: CODESSA ; Kier flexibility index (1.25 to 16.57)
DPSA1: CODESSA; difference in positive and negative partial surface areas (−100.41 to 563.06)
Av. valency: CODESSA; average valency (3.63 to 4.47)
Rel. no. O atoms: CODESSA; relative number of oxygen atoms (0 to 0.50)
S_N2
SSP (−0.377 to 3.700)
S14: winMolconn; atom level E-state for atom 14 (−3.234 to 11.013)
$SsCH_3$: winMolconn; E-state forCH_3 carbon atoms (0 to 7.701)
xvp9: winMolconn; ninth order valence path molecular connectivity (0 to 0.506)
eaC2C3a: winMolconn; bond-type E-state for single bond between unsubstituted carbon and carbon with three aromatic neighbors (0 to 12.937)
FASA-: MOE; fractional accessible surface area of all atoms with negative partial charge (0.103 to 0.673)
PEOE_VSA_FPOS: MOE; fractional positive van der Waals surface area (0.265 to 0.775)
MNDO_HOMO: MOE; energy of the highest occupied molecular orbital calculated using the MNDO Hamiltonian [MOPAC] (−12.102 to −8.237)
Acyl Transfer
SSP (0.075 to 3.860)
HS14: Winmolconn; hydrogen atom level HE-state for hydrogen atom 14 (0 to 2.749)
HS16: Winmolconn; hydrogen atom level HE-state for hydrogen atom 16 (0 to 2.711)
HS17: Winmolconn; hydrogen atom level HE-state for hydrogen atom 17 (0 to 1.514)
HS29: Winmolconn; hydrogen atom level HE-state for hydrogen atom 29 (0 to 2.898)
Oxidation Potential
SSP (−0.980 to 0.695)
vsurf_DD12: MOE; contact distances of vsurf_DDmin (3 descriptors) (0.500 to 7.697)
vsurf_DD23: MOE; contact distances of vsurf_DDmin (3 descriptors) (0.500 to 6.819)

It is also important that there should be no high pairwise correlations between the various descriptors incorporated into a QSAR; otherwise, the statistics could be flawed.[23] Using a cutoff point of $r = 0.9$,[48] we found no such high correlations between any of the descriptors used in each QSAR.

RESULTS AND DISCUSSION

The QSARs that we developed for each mechanistic category, as well as that for all 204 chemicals together, are given in Table 2.

Explanations of the descriptors are given in Table 3. We recognize that, in some cases, the explanations are sparse, but descriptor software is frequently short on detail. Table 3 also includes the ranges of SSPs and descriptor values in each mechanistic category, as an indication of the applicability domains of each category. The SSPs cover a very wide range of potency, ranging from weak to strong or extreme, save for the oxidation potential category, in which the range is from weak to moderate (EC3 values from 89 to 5%).

For each category with adequate numbers of chemicals, with two exceptions, we were able to formulate good QSARs with good internal and external validations. The first exception is the Schiff base category, for which we could obtain a QSAR that, while acceptable, was not good enough for our purposes, namely, to provide QSAR models that can offer good prediction. However, by combining the Schiff base chemicals with the five in the pro-Schiff base category, we were able to develop a QSAR with good internal and external predictive abilities. The second exception is the acyl transfer category, for which a good model could not be developed using all 23 acyl transfer chemicals, owing to one chemical, C11 azlactone, being a pronounced outlier. Several azlactones, with alkyl chains ranging from C4 to C19, have been tested in the LLNA (see Table 1), and they appear to

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Chem. Res. Toxicol. 2015, 28, 1975−1986

Table 4. Comparison of Statistical Quality of Full Dataset QSARs

category	all	MA	pMA	SB	SB + pSB	S_N2	acyl	OxPot
equation	1	2	5	8	11	14	17	20
n	204	45	32	35	40	45	22	11
descriptors	6	6	5	7	7	7	4	2
R^2	0.496	0.856	0.858	0.837	0.850	0.852	0.921	0.930
R^2_{adj}	0.480	0.834	0.831	0.795	0.817	0.823	0.902	0.912
Q^2	0.459	0.793	0.790	0.644	0.781	0.796	0.886	0.856
SE	0.689	0.358	0.349	0.259	0.233	0.381	0.304	0.156
F	32.4	37.8	31.3	19.9	25.9	30.3	49.5	52.8

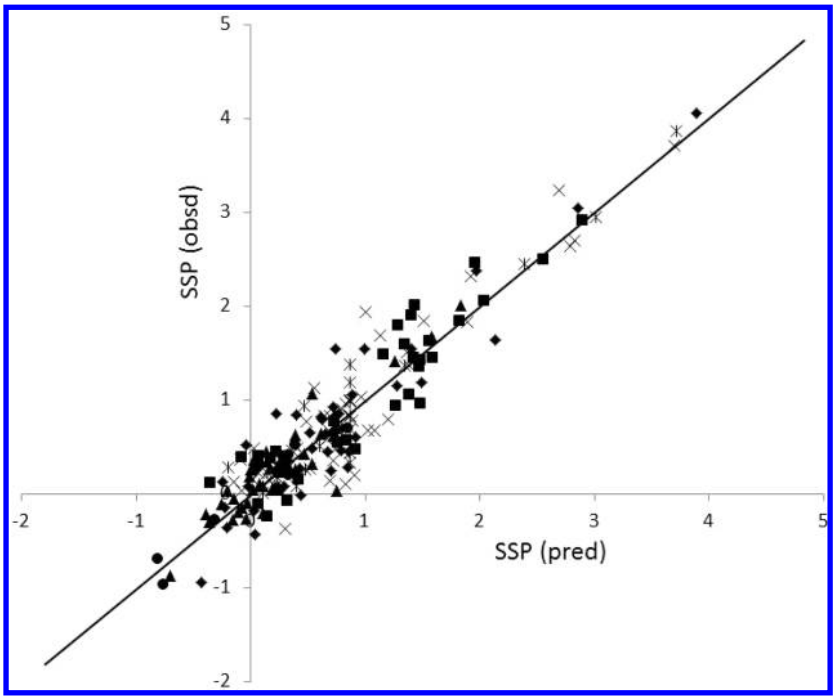

Figure 1. Observed vs predicted SSP values for all 196 chemicals. Black diamond = Michael addition; black square = pro-Michael addition; black triangle = Schiff base + pro-Schiff base; cross = S_N2; asterisk = acyl transfer; black circle = oxidation potential.

fall into two groups, separated by an activity cliff.[49] Shorter-chain-length azlactones (C4−C9) are quite potent, with EC3 values between 1 and 3%, whereas longer-chain homologues (C15−C19) are much weaker, with EC3 values of about 20%. This presumably reflects a change in the rate-determining step (possibly mass transfer) becoming rate-limiting for azlactones with high hydrophobicity.[50] Our model is able to make this distinction, but it appears that the C11 homologue, which is structurally between these two subsets and should belong to the low-potency subset, is treated by our model as belonging to the high-potency subset. When the C11 azlactone was removed, a good QSAR model was obtained (Table 2, eqs 17 and 18). The statistical quality of all the models can be seen from Table 4.

It would, of course, have been possible to increase R^2 and SE values for most of the models by increasing the number of descriptors incorporated. However, as we have pointed out elsewhere,[22] "the principle of Occam's razor (principle of parsimony) applies here: 'One should not increase beyond what is necessary the number of entities required to explain anything'. We suggest that five or six descriptors are generally the maximum that one should generally use in a QSAR/QSPR, partly because it is difficult to comprehend the mechanistic significance of large numbers of descriptors". We were surprised but very pleased that the two categories with the smallest number of chemicals (acyl transfer and oxidation potential) could, nevertheless, allow good QSARs to be developed. In fact, the latter category yielded the best QSAR of all.

The observed SSPs for all 195 skin sensitizers used in our modeling were correlated with the cumulative SSP values calculated from each appropriate local mechanistic domain QSAR, and as expected, a very good correlation was found:

$$\text{SSP (observed)} = 0.000 + 1.000\ \text{SSP (predicted)} \quad (22)$$

$$n = 195,\ R^2 = 0.884,\ Q^2 = 0.882,\ \text{ICC} = 0.939,$$
$$\text{SE} = 0.296,\ F = 1471$$

A graphical representation of these results is shown in Figure 1.

All test sets yielded very good predictions, fortuitously with all R^2 values being higher than those of the full and training set QSARs.

The correlation between observed and predicted SSP values for all 37 test set chemicals was found to be

$$\text{SSP (obsd)} = -0.070 + 1.002\ \text{SSP (pred)} \quad (23)$$

$$n = 37,\ R^2 = 0.947,\ Q^2 = 0.940,\ \text{ICC} = 0.971,$$
$$\text{SE} = 0.209,\ F = 627.3$$

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Chem. Res. Toxicol. 2015, 28, 1975−1986

The overall ICC of 0.971 for all test set results indicates that the test set results for all mechanisms were valid. This can also be seen from Figure 2.

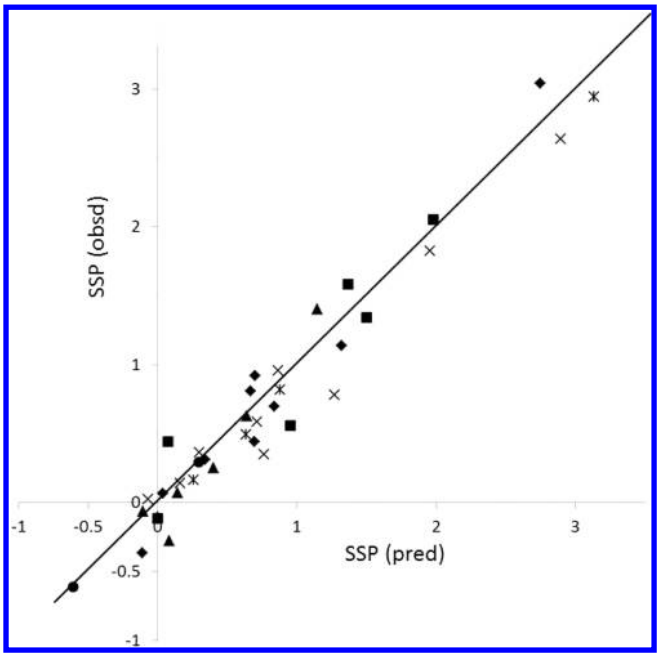

Figure 2. Observed vs predicted SSP values for all 37 test set chemicals. The 45° line on the graph is virtually indistinguishable from that of eq 23. Black diamond = Michael addition; black square = pro-Michael addition; black triangle = Schiff base + pro-Schiff base; cross = S_N2; asterisk = acyl transfer; black circle = oxidation potential.

The QSAR derived for the complete data set of 204 active chemicals, covering all of the reaction mechanistic categories, is very much inferior to any of the QSARs for the individual mechanistic categories (Table 2), and the descriptors found to model the potency best are different for each mechanistic category, as can be seen from Table 3. These findings reinforce the argument that, for skin sensitization, modeling reaction mechanistic domains/categories has more realistic prospects of success than attempting a global model.

The model obtained for Schiff base chemicals was not very good (n = 35, R^2 = 0.837, Q^2 = 0.644, SE = 0.259, F = 19.9). However, inclusion of the five pro-Schiff base chemicals improved the model considerably (n = 40, R^2 = 0.850, Q^2 = 0.781, SE = 0.233, F = 25.9).

It has been found that depending on the reaction mechanism of the protein-binding step there are different relationships between model reactivity parameters and potency.[50−52] This is argued to be because, depending on the reaction mechanism, relative reactivities toward the several nucleophilic protein sites will differ. Thus, for example, the Schiff category chemicals probably sensitize via reaction with amino groups of proteins, whereas the Michael acceptor category chemicals probably sensitize via reaction with protein thiol groups. Even where compounds from two different mechanistic categories sensitize via reaction with the same type of protein nucleophile, the proportionality between the *in cutaneo* reactivity and reactivity determined in a model cannot be assumed to be the same. This should apply irrespective of whether the model reactivity is based on experimental data with model nucleophiles, on classical linear free energy relationship indices based on Hammett and Taft substituent constants, on quantum mechanical indices such as activation energy,[53] or on combinations of less transparent descriptors such as those used here. Furthermore, for some reaction mechanistic categories (Schiff base,[11,50] S_N2, and acyl transfer[50]), potency has been found to be dependent not only on reactivity but also on hydrophobicity, whereas for others (Michael acceptors,[12] S_NAr electrophiles[13]), reactivity parameters alone can give good models for potency. It has already been mentioned that many descriptors are difficult to interpret. Those selected for the Michael addition category suggest that reactivity and surface area, and perhaps especially hydrophobic surface area, enhance skin sensitization potency. For pro-Michael addition, several descriptors represent hydrogen bonding, although there does not appear to be a consistent pattern; for example, SssNH has a positive coefficient, whereas that for vsurf_HB7 is negative.

From eq 8 (Table 2), it can be seen that for Schiff base chemicals polarity and molecular flexibility increase potency. There are also some specific atom effects (S7 and S10), although, as the nature of those atoms is not known, no interpretation of those effects can be made. The situation is somewhat clearer for the combined Schiff base and pro-Schiff base model (eq 11; Table 2), with hydrogen bonding (represented by HS6, E_sol and possibly DPSA1) being important for potency, together with molecular shape (dx2 and Kier FI).

S_N2 chemicals appear to require hydrophobicity ($SsCH_3$, eaC2C3a) for potency, although descriptors representing both negative and positive surface area also have positive coefficients. Electron-donating ability (MNDO_HOMO) decreases potency, which is to be expected since Michael reactivity is dependent on the electron deficiency of the double or triple bond.

Acyl transfer appears to be highly dependent on hydrogen bonding, as all four descriptors are E-state values for different hydrogen atoms. Finally, oxidation potential appears possibly to be dependent on molecular shape as well as the location of interacting atoms or groups, as contact distances are important (vsurf_DD12, vsurf_DD23).

It should be noted that while hydrophobicity (represented in many QSAR studies as log P, the logarithm of the octanol−water partition coefficient) is not specifically selected as a descriptor in any of our models it is a composite descriptor with components of polarity, polarizability, hydrogen bonding, and molecular size,[54] so our models are not incompatible with previous studies[11,50] that found hydrophobicity to be important.

On the basis of the above perspective, we have shown that quantitative predictive models for sensitization potency can be derived by (i) assigning chemicals to reaction mechanistic domains, (ii) determining appropriate reactivity parameters and (if necessary) hydrophobicity within a mechanistic domain, and (iii) deriving regression-based quantitative mechanistic models and using these to estimate the potency for untested chemicals. This chemistry-based approach can already enable potency to be predicted for many chemicals.[51] The findings presented here strongly reinforce the argument that assignment of chemicals to their reaction mechanistic domains (categories) is an essential step before attempting to predict potency by *in chemico* or *in silico* approaches.

All of the QSARs reported here satisfy all or almost all of the OECD Principles for the Validation of (Q)SARs.[55] The work described here offers one solution to the vital need, emphasized by Basketter et al.,[56] for information on the potency of identified skin sensitizers in order to permit risk assessment.

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CONCLUSIONS

Using in-house expertise, we have allocated 204 skin-sensitizing chemicals to their respective mechanistic categories and then developed good QSAR models, with good predictive ability, for chemicals in seven out of 10 categories. Only one chemical had to be omitted as an outlier, and an explanation is provided for that omission. Data on too few chemicals were available to allow QSAR modeling for three categories, namely S_N1, pro-S_N2, and S_NAr. The QSARs reported here can be used, either on their own or as part of a weight-of-evidence approach, in risk assessments of skin sensitization.

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Notes

The authors declare no competing financial interest.

DEDICATION

Professor Alan Katritzky passed away on 10 February, 2014. We dedicate this article to his memory.

ABBREVIATIONS

Ac, acyl transfer; CAS, Chemical Abstracts Service; EC3, the concentration (g/100 mL) that induces a 3-fold increase in local lymph node proliferative activity relative to controls; *F*, coefficient of variance (Fisher statistic); ICC, intraclass correlation coefficient; LLNA, murine local lymph node assay; MA, Michael addition; MLR, multiple linear regression; MW, molecular weight (relative molecular mass); OxPot, oxidation potential; p-MA, pro-Michael addition; OECD, Organisation for Economic Cooperation and Development; *p* value, probability that a descriptor is there by chance; p-SB, pro-Schiff base; p-S_N2, pro-bimolecular aliphatic nucleophilic substitution; Q^2, cross-validated coefficient of variation (leave-one-out procedure); QSAR, quantitative structure−activity relationship; *r*, correlation coefficient; R^2, coefficient of variation; R^2_{adj}, coefficient of variation adjusted for degrees of freedom; REACH, Registration, Evaluation, Authorisation and restriction of Chemicals; SE, standard error of estimate; SB, Schiff base; S_N1, unimolecular aliphatic nucleophilic substitution; S_N2, bimolecular aliphatic nucleophilic substitution; S_NAr, bimolecular aromatic nucleophilic substitution; SSP, skin sensitization potency

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