The ECVAM International Validation Study on *In Vitro* Tests for Acute Skin Irritation: Selection of Test Chemicals

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Summary — The ECVAM-funded skin irritation validation study (SIVS) was initiated in 2003, with the aim to evaluate whether the EpiDerm™, EPISKIN™ and the SIFT alternative methods were able to reliably identify skin irritant and non-irritant chemicals, and could therefore be candidates for replacing the rabbit Draize test for skin irritation. The primary goal of the study was to evaluate the predictive capacity of the assays with regard to the EU classification system, which employs the risk phrases, "R38", for skin irritants, and "no label" for non-irritants. A secondary objective was the retrospective analysis of the data, to assess whether the *in vitro* tests would be able to discriminate between strong irritants (category 2), mild irritants (category 3) and non-irritants (no category), as defined by the OECD and United Nations proposal for a Globally Harmonised System (GHS) for the classification and labelling of dermal irritancy. A Chemicals Selection Sub-Committee (CSSC) was appointed to identify test chemicals to be used in the SIVS, for which existing, high quality *in vivo* data were available, with which to correlate the *in vitro* measurements. Since chemicals from the European Centre for the Ecotoxicology and Toxicology of Chemicals (ECETOC) database of reference chemicals for skin irritation/skin corrosion had been extensively used in preceding studies, the CSSC made use of novel sources for potential test chemicals. The first source of chemicals screened was the New Chemicals Database (NCD), which is the central archive within the EU notification scheme for 'new' commercial chemicals. Data registered in the NCD originate from standard assays, submitted in compliance with the legislation which regulates the marketing of industrial chemicals, and are subject to quality assurance by the competent authorities of the EU Member States. In addition, to obtain 'existing' chemicals which were readily available from major manufacturing and/or distribution sources, additional databases were surveyed, such as the Toxic Substance Control Act (TSCA) database maintained by the US Environmental Protection Agency (EPA), and the ECETOC database, with the exclusion of the chemicals used in the previous optimisation and prevalidation phases. A total of approximately 3500 chemicals from the NCD and 1600 from the additional databases were screened. Pre-determined selection criteria were applied, primarily to ensure the quality of the *in vivo* data and the practicability of their use in testing. Overall, the number of chemicals fulfilling the CSSC selection criteria was found to be limited, particularly in the case of GHS category 2 chemicals. However, a total set of 60 chemicals were selected and proposed to the Management Team of the SIVS for independent coding and supply to the participating laboratories. The selected chemicals: i) represented statistically justified sample sizes for distinguishing R38 from no-label chemicals; ii) provided a balanced representation of the three GHS categories, to allow for the *post hoc* evaluation of the performance of the assays for that classification system; and iii) acknowledged, to a certain degree, the large prevalence known to exist for chemicals which have oedema and erythema scores of 0. The selected chemicals represented a variety of molecular structures, functional chemical groups, and effect and use categories, as well as a wide range of physico–chemical properties. They represented a challenging set of chemicals, relevant to current industrial commerce, with which to validate the alternative methods.

Key words: *Draize skin test, ECVAM, ECETOC database, EpiDerm, EPISKIN, GHS, New Chemicals Database, SIFT, skin irritation, test chemicals, TSCA database, validation.*

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1. Introduction

Currently, dermal irritation is assessed by the potential of a given substance to cause erythema/eschar and/or oedema after a single topical application on rabbit skin, in accordance with the Method B.4 of Annex V to EU *Directive 67/548/EEC* or OECD Test Guideline (TG) 404 (1, 2). However, current European cosmetics and chemicals legislation prescribe the use of alternative methods to animal testing (3). In particular, the 7th Amendment to the EU Cosmetics Directive, *Directive 76/768/EEC*, decrees a complete ban on animal testing for cosmetic ingredients as soon as alternative non-animal methods have

been endorsed as validated by ECVAM and adopted into the EU legislation, with a maximum period of implementation until March 2009, when the animal testing ban will be enforced, regardless of the availability of alternative test methods (4, 5).

In order to identify *in vitro* tests capable of identifying skin irritants and non-irritant chemicals, several prevalidation and optimisation efforts on *in vitro* alternatives have taken place over the last decade (6). In particular, an ECVAM prevalidation study was conducted during 1999 and 2000, in which five promising *in vitro* methods were evaluated, namely, EpiDerm™, EPISKIN™, Prediskin™, the non-perfused pig ear model, and the *in vitro* mouse skin integrity function test (SIFT). The conclusion from this study was that, although the reproducibility of the two human skin model tests (EpiDerm and EPISKIN) and of the SIFT test was considered acceptable, their predictive capacities needed further improvement. ECVAM therefore recommended the optimisation of the protocols and prediction models (PMs) of the three assays, as well a sharing of the experience gained with the EpiDerm and EPISKIN human-skin models, in order to develop a common protocol (7). Subsequently, refinements were made to the EpiDerm and EPISKIN protocols, and to the SIFT assay, so that their optimised test protocols and/or PMs finally met the criteria for inclusion in a formal validation study (8–11).

An ECVAM-funded skin irritation validation study (SIVS) was initiated in 2003. The aim of the study was to evaluate whether the EpiDerm, EPISKIN and the SIFT alternative methods were able to reliably identify skin irritant and non-irritant chemicals, and could therefore be candidates for replacing the rabbit Draize test for skin irritation. An international Management Team (MT) coordinated the study, which was divided in two phases of experiment: a first phase to optimise and confirm the study protocols by the lead laboratories; and a second phase to determine the reproducibilities and predictive capacities of the assays in three laboratories for each assay. In both phases, the assays were evaluated by testing a set of preselected substances, in a blind manner with coded identities.

A Chemicals Selection Sub-Committee (CSSC) was appointed to identify test chemicals that could be used in the study. Since chemicals from the European Centre for the Ecotoxicology and Toxicology of Chemicals (ECETOC, Brussels, Belgium) database of reference chemicals for skin irritation/skin corrosion (12) had been extensively used in the preceding studies, the CSSC also made use of other sources of potential test chemicals.

The leading criterion for the selection of chemicals was the availability of existing, high quality *in vivo* data, with which to correlate the *in vitro* data. For this reason, the first source of chemicals screened was the New Chemicals Database (NCD), which is managed by the European Chemicals Bureau (ECB, a partner unit of ECVAM at the European Commission Joint Research Centre). The NCD is the central archive within the EU notification scheme for 'new' commercial chemicals, defined as substances introduced to the EU industrial market after 1981. The files registered in the NCD are subject to regulator review and approval. Integral to comprehensive hazard assessment obligations, skin irritation testing is requisite according to regulatory test methods, and quality standards such as Good Laboratory Practice (Annex V of *Directive 67/548/EEC*; 1, 13). Notification files also register substance origin (manufacturer, notifier, importer), industry uses, and other hazard classifications assigned by the Competent Authorities of EU Member States. Periodically, hazard classifications agreed by formal consent among the Competent Authorities for schedules of chemicals, are made officially, in updates of Annex I to *Directive 67/458/EEC*. The NCD therefore provided a primary operational source for the selection of candidate chemicals, supported by reviewed, standardised and quality compliant *in vivo* data.

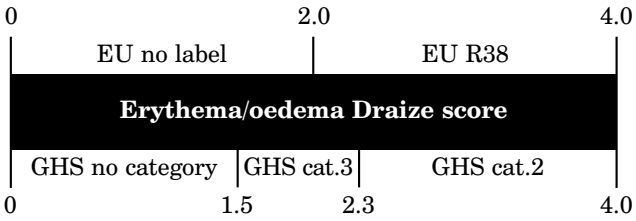
Supplementary to the NCD, additional 'existing' candidate chemicals, which were readily available from major manufacturing and/or distribution sources, were identified with supporting *in vivo* data, from: a) the TSCA (Toxic Substance Control Act) database maintained by the US Environmental Protection Agency (EPA, Washington, DC, USA); b) the ICCVAM public call for the submission of dermal irritancy chemicals and protocol information/ test data, published in the US *Federal Register* (14) as described below; and c) the ECETOC database, excluding those chemicals used in the previous optimisation and prevalidation phases.

2. Chemical Selection Strategy

The primary goal of the SIVS was to evaluate whether the EpiDerm, EPISKIN and the SIFT assays could reliably identify skin irritants labelled "R38" according to the EU risk phrase, and non-irrritants with "no label", as defined by EU *Directive 67/548/EEC* (15). A secondary objective was the retrospective analysis of the data, to assess whether the *in vitro* tests would be able to discriminate between strong irritant (category 2), mild irritant (category 3) and non-irritant (no category) chemicals, as defined by the OECD and United Nations proposal for a Globally Harmonised System (GHS) for the classification and labelling of dermal irritancy (16). The differences between the two classification systems are illustrated in Figure 1.

The number of chemicals required was determined statistically by sample size calculation, as fol-

Figure 1: The erythema/oedema Draize score ranges which define the EU and GHS classification systems for skin irritation



The scores refer to at least two out of three animals, or the mean value in cases where more than 3 animals are used.

lows. Sensitivity, i.e. the proportion of chemicals correctly predicted as skin irritants, and specificity, i.e. the proportion of chemicals correctly predicted as non-irritants, were defined as the primary parameters to be estimated within the validation study. To simplify the calculation, the independence of these two parameters, as well as the independence of the classification of a given chemical from the classification of another chemical, were assumed. Taking into account the experience of the laboratories with the assays, and a minimum requirement in terms of predictive capacity, a specificity and a sensitivity of at least 75% were expected from the assays. Based on this assumption, it was estimated how large the sample size for a binomial proportion had to be, in order to have a lower limit of a one-sided confidence interval significantly larger than 0.5, with a significance level of 0.05 and with a power of at least 0.75, as generalised by Flahault *et al.* (17). By using the software package, S-Plus 6.1 (Insightful, Seattle, WA, USA), it was determined that a sample size of 26 chemicals for each class of irritancy, i.e., skin irritants and non-irritant compounds, would be necessary.

The prevalence of skin irritants among the chemicals registered within the NCD and listed in the ECETOC database was further evaluated. Of 3121 chemicals surveyed, less than 8% caused skin irritation in rabbits which would require a R38 label, while 64% did not result in any oedema and erythema irritation scores (for details of this analysis, see 18). Although the limited feasibility of fully implementing such a prevalence in the study design was acknowledged, its consideration in the assessment of the *in vitro* tests was advised by the MT.

On this basis, the MT decided on the testing of a total of 60 chemicals in the SIVS, including at least 26 R38 and 28 no-label chemicals. In practice, 20 chemicals were to be tested in Phase 1, then re-coded and included among the 60 chemicals to be tested in Phase 2.

In order to i) achieve a balance between irritants and non-irritants, according to the EU classification, ii) to have representation of the three GHS categories, and iii) to acknowledge, to a certain degree, the large percentage of registered chemicals which have oedema and erythema scores of 0 (18), the CSSC aimed at selecting chemicals which presented a distribution of irritancy scores, as illustrated in Figure 2.

3. Selection of 'New' Chemicals Registered in the NCD

3.1 Primary extraction of NCD chemicals

At the time of the chemicals selection, in the spring of 2003, the NCD contained approximately 5600 notifications, concerning about 3500 substances. The following exclusion criteria were applied in a primary survey and extraction of data:

1. Substances notified before 1995 were initially excluded, and the search was focused on notifications from the last decade, for which complete electronic data files would be assured, and where contacting companies for the supply of substance sample material would be facilitated.

2. For multiple dossiers concerning the same substance, repeat notifications were excluded, and only data from the file leader, usually the original notifier, were extracted.

3. Substances marketed at quantities below 0.1 tonnes/year, for which skin irritation testing is not a regulatory requirement, were excluded.

4. Skin corrosives were excluded, as the primary focus was on skin irritation.

5. Adsorbents, gases and vapours were excluded for technical reasons linked to the test protocols being validated.

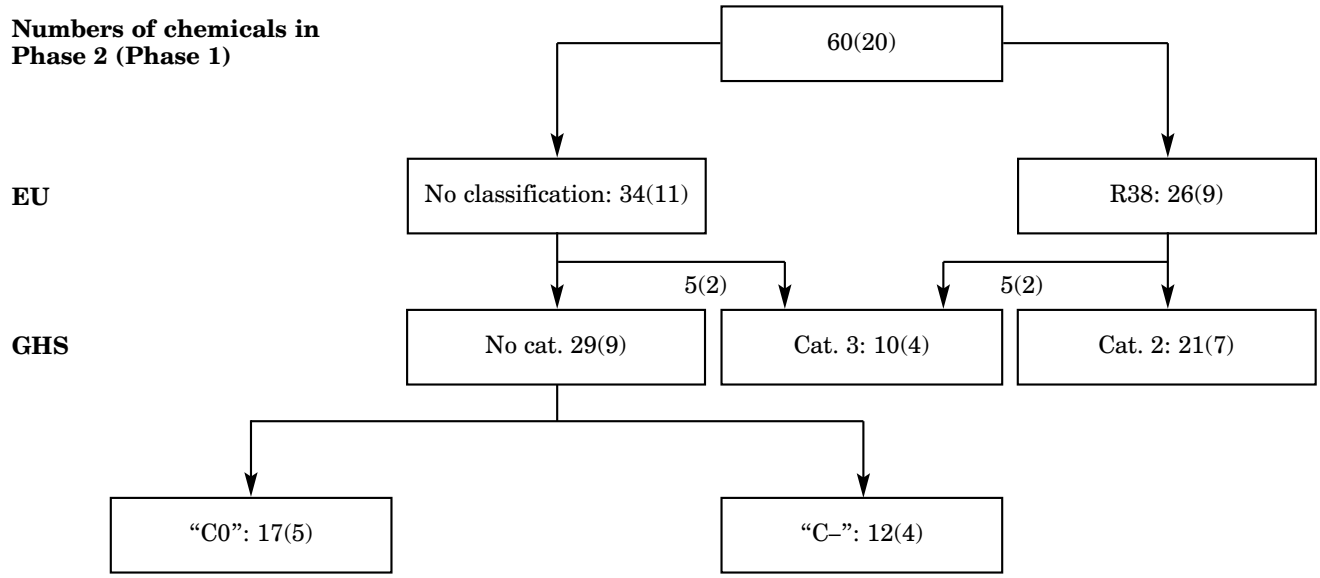
Applying these exclusion criteria, the NCD survey yielded 1307 substances, comprising 132 skin irritants (R38) and 1175 non-irritants (no label; Table 1), based on skin irritant classifications registered by EU Competent Authorities. The following information on these substances was then extracted:

— substance identification and molecular structure;

— physical state (solid/liquid);

— purity (typical %, lower limit %, upper limit %);

Figure 2: The chemical selection strategy, with reference to EU and GHS classification systems



"C0" represents chemicals with oedema and erythema scores of 0; "C–" represents GHS non-classified chemicals with oedema and/or erythema scores different from 0.

— skin irritation data (*in vivo* erythema and oedema scores);
— classification and labelling;
— whether a mixture (Y/N);
— molecular weight (MW), including components in mixtures;
— octanol–water partition coefficient (log K_{ow});
— water solubility;
— vapour pressure;
— melting point and boiling point;
— desired effect and use categories; and
— name(s) of producer and/or notifier (including country of origin).

3.2 Secondary reduction of NCD chemicals

A secondary data reduction applied the following quality criteria on the extracted physico–chemical properties:

1. Substances with no known purity, or with a typical purity of less than 94%, were disregarded.

2. According to the regulatory definitions, single substances can comprise reaction mixtures of unseparated components, such as isomers or generically similar molecules. Substances with more than three components (or more than four components for isomeric mixtures) were excluded.

Table 1: Selection of SIVS candidate chemicals from the NCD, partitioned according to the EU and GHS classification and labelling systems[a]

	EU R38		EU no label		
	GHS cat. 2	GHS cat. 3	GHS cat. 3	GHS no cat.	Total
Primary extraction	132		1175		1307
Secondary reduction	28	59	27	218	332
Selection refinement	11	21	7	87	126
Selected NCD substances obtained from suppliers	**7**	**11**	**4**	**15**	**37**

[a]*The criteria for primary extraction, secondary reduction and selection refinement are described in the text.*

3. Complex mixtures, substances with unidentified components, and substances with uncertain component proportions, were excluded.

4. Substances with no known MW, with a MW over 1000, or with a range of MWs (e.g. polymers), were excluded.

No exclusion criteria were applied on the basis of water solubility, log K_{ow}, vapour pressure, boiling point or melting point, in order to deliberately allow for variability in these physico–chemical characteristics. In addition, no exclusion criteria were applied on the basis of other topical irritation, namely, skin sensitisation and eye irritation, again to allow scope for variability.

Following the application of the secondary elimination criteria, 845 candidate chemicals remained from the primary list of 1307, including 87 irritants and 758 no-label chemicals. The *in vivo* data (erythema and oedema scores) for the short-listed substances were reviewed, with irritant classifications being assigned according to the GHS classification scheme. Due to an excessive number of GHS non-irritant substances (731), a pragmatic refinement gave preference to irritant substances notified by the same suppliers, and to substances indicated for use as cosmetic ingredients. Based on this refinement, a total of 218 non-irritants were short-listed (see Table 1).

3.3 Refinement of the selection of NCD chemicals

The short-listed skin irritants and non-irritants were then further screened for properties which would cause practical difficulties in testing, as well as for any classification inconsistencies. The following refinement criteria were applied:

1. Particularly hazardous chemicals, such as carcinogens and explosives, were eliminated for safety reasons.

2. Chemicals likely to present testing difficulties, such as those with hydrolysing properties, polymerising tendencies, or samples available only as preparations, were excluded.

3. Chemicals no longer in production (reported by a regulatory authority) were excluded.

4. Chemicals having classifications inconsistent with the Draize test scores were disregarded. These comprise chemicals classified on the basis of a non-standard method, read-across, or persistent effects.

It is important to note that, for chemicals classified on the basis of persistence, a parallel study was initially foreseen, in which the lead laboratories would test them with the *in vitro* methods under validation. However, out of all the chemicals screened, only three substances were found to be classified as R38 on the basis of persistence of effects. In addition, only one could be obtained from the contacted suppliers, and insufficient information was available to allow its inclusion in the study. As a consequence, the proposed parallel study could not take place.

As a result of the application of the selection criteria, 27 R38 and 94 no-label chemicals were selected. As the number of skin irritants barely represented the minimum sample size required, and since it was anticipated that there might be problems with the manufacturer/supplier of some of them, the CSSC extended the scope of the primary NCD survey, by searching for R38 substances notified prior to 1995. The primary extraction resulted in the identification of a further 54 chemicals, of which five R38 substances met the CSSC's secondary reduction and selection refinement criteria.

A total of 126 substances were finally selected by the CSSC from the NCD as suitable test materials for the SIVS, comprising 32 skin irritants and 94 non-irritants (Table 1). For quality control, it was reconfirmed that the assigned classifications and labelling (R38 or no label) proposed by the Competent Authorities and/or those published in Annex I of *Directive 67/548/EEC* (19), could be derived from the rabbit Draize scores for these substances.

3.4 Suppliers contact and confidentiality issues

A total of 58 notifiers and/or producers were identified for supplying the 126 selected substances. In cases where contact addresses were obsolete, updated information was sought, by invoking assistance of the European Chemical Industry Council (CEFIC) and the European Federation for Cosmetic Ingredients (EFfCI). Subsequently, 47 producers and/or notifiers were contacted for the supply of 115 of the 126 selected substances, and sample materials were requested. Responses were eventually received from 30 companies, of which 25 were able to cooperate (Table 2) by providing a total of 37 test samples, comprising 18 skin irritants and 19 non-irritants (Table 1).

During the course of the study, the collaborating companies (Table 2) were requested to release the identities (IUPAC names, CAS numbers, and structural formulae) of chemicals registered in the NCD as confidential proprietary information. Of the 25 suppliers, 24 agreed to releasing this commercially sensitive information, representing data for 35 of the 37 substances. Furthermore, agreement to release the skin irritation classifications was confirmed with these suppliers. However, it was agreed

Table 2: Supplier companies (manufacturer/notifier/importer) contributing samples of test substances selected from the NCD, and used in the SIVS

Supplier	Location	
Akzo Nobel–Diosynth	Oss	The Netherlands
AstraZeneca	Södertälije	Sweden
Basell	Ferrara	Italy
BASF	Ludwigshafen	Germany
Cambrex	Cork	Ireland
Clariant	Paris	France
DSM Nutritional Products	Saint-Louis	France
Esteve Quimica	Barcelona	Spain
Evonik Goldschmidt GmbH	Barcelona	Spain
	Essen	Germany
Firmenich	Geneva	Switzerland
Givaudan	Vernier	Switzerland
	Argenteuil	France
Huntsman Advanced Materials	Basel	Switzerland
Industries Chimiques de Mulhouse–Dornach	Mulhouse	France
International Flavors & Fragrances	Hilversum	The Netherlands
	Co. Louth	Ireland
		Spain
	Union Beach, NJ	USA
Janssen Pharmaceutica	Beerse	Belgium
Kao Corporation	Barcelona	Spain
Laboratori Fitocosmesi e Farmaceutici	Milan	Italy
Nisseki Chemical	Passadena, TX	USA
Omnichem	Wetteren	Belgium
PFW Aroma Chemicals	Barneveld	The Netherlands
Promerus	Brecksville, OH	USA
Rhodia Organica	Saint Fons	France
SACI–CFPA	Paris	France
Safepharm Laboratories	Derbyshire	UK
Symrise	Holzminden	Germany

that the individual suppliers would remain anonymous, and would not be associated with any specific substances, nor would the corresponding Draize scores registered for the NCD substances be disclosed. Nevertheless, the CSSC itself had full access to the *in vivo* observations and individual scores for the NCD chemicals.

Permission was not obtained for the disclosure of the identities of the two remaining substances. Consequently, the *in vitro* results obtained for these two substances were not considered in the final evaluation of the SIVS.

4. Selection of Additional Chemicals

Due to the shortfall in availability of the NCD chemicals and the need to include chemicals which were readily available from major manufacturing and/or distribution sources, complementary 'existing' chemicals were selected for Phase 2 of the SIVS, through the recourse to additional databases.

4.1 Collaboration with ICCVAM–NICEATM

The NTP Interagency Center for the Evaluation of Alternative Methods (NICEATM, NIEHS, Research Triangle Park, NC, USA) and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM, MIEHS, Research Triangle Park, NC, USA), in collaboration with the ICCVAM Dermal Corrosivity and Irritation Working Group, provided the CSSC with two lists of candidate chemicals, based on the following sources: data submitted to the EPA in accordance with the TSCA, and data submitted in response to a public call for Draize dermal irritation data (14).

The TSCA database contained information on 3400 industrial chemicals, of which 2400 could be

obtained through a major manufacturing and/or distribution source. A total of 1312 reports were reviewed by ICCVAM–NICEATM, out of the 1905 files received, and 42 reports were found to contain dermal irritation data which the indications suggested would be potentially useful to the SIVS.

The public call for data on Draize dermal irritation was launched via a US *Federal Register* notice, published in July 2003 (14). It requested data on commercially-available chemicals tested for skin irritancy in rabbits by using current standardised testing methods, and data on skin irritancy from human studies and from human post-marketing or occupational exposure or surveillance evaluations. ICCVAM–NICEATM received data from three different sources: 1) data on 124 substances were received from the Research Institute of Fragrance Materials (RIFM, Hackensack, NJ, USA); 2) data on six substances were received from the US Cosmetic, Toiletry, and Fragrance Association (CTFA, Washington, DC, USA); and 3) data on six substances were received from the European Isocyanate & Polyol Producers Association (ISOPA, Brussels, Belgium). In addition, the CTFA provided ICCVAM with the 2003 Cosmetic Ingredient Review (CIR) compendium, which contains summaries of toxicological studies (animal and human) for 1119 cosmetic ingredients. A total of 45 complete reports were received from the CIR database, from which NICEATM identified one chemical with dermal irritation data which indicated that it would be potentially useful for the SIVS.

Overall, 43 files were provided to ECVAM by ICCVAM–NICEATM, with detailed information on purity and individual rabbit Draize irritation test scores. Information from the 43 files on six substances was found to comply with the standard protocols for dermal irritation, and to fulfil the CSSC secondary reduction and selection refinement criteria, as described above. The six substances, representing two skin irritants (R38) and four non-irritants (no label, according to the EU classification and labelling system) were included in Phase 2 of the SIVS.

4.2 The ECETOC Database

The ECETOC Reference Chemicals Database for skin irritation/skin corrosion contains a total of 129 chemicals, for which detailed *in vivo* data from dermal irritation studies are available (12). The CSSC applied the secondary reduction and selection refinement criteria to 34 eligible substances that had not previously been tested in the prevalidation and protocol optimisation phases, prior to the organisation of the SIVS. This resulted in a shortlist of 14 chemicals, which were then complemented with an additional five chemicals which had been used in previous protocol optimisation and prevalidation phases, in order to provide the 60 chemicals required for Phase 2 of the SIVS. This total of 19 chemicals included 7 skin irritants (R38) and 12 non-irritants (no label, according to the EU classification and labelling system).

4.3 Considerations concerning the additional chemicals

When selecting additional chemicals from readily-available commercial sources, the CSSC confirmed that the *in vivo* test protocol used was compliant with the EU and OECD standards and guidelines (1, 2), and that individual rabbit Draize scores were available for each of at least three rabbits, or that at least the mean scores were reported when more than three animals were used.

As only two of the additional (non-NCD) chemicals used in the SIVS have an official hazard classification assignment (i.e. as published in Annex I to *Directive 67/548/EEC*), implicit classifications were derived, based on the reported *in vivo* Draize scores. Commercial classifications provided by the chemical suppliers were not considered, as these were not traceable nor standardised, as were the official classifications.

The physico–chemical properties of the non-NCD chemicals were obtained from the available documented sources, including on-line databases and supplier safety data sheets. However, the information retrieved was not always complete, and was of an uncertain level of quality, in contrast to the chemicals registered in the NCD.

5. Results

In total, approximately 3500 'new' commercial chemicals registered in the NCD, and 1600 'existing' chemicals recorded in alternative databases, such as the TSCA, CIR and ECETOC databases, were screened. Only a limited number were found to fulfil the CSSC selection criteria, but it was pos-

Table 3: Distribution of SIVS Phase 1 chemicals selected from the NCD, according to the EU and GHS classification and labelling systems

		NCD
R38	GHS cat. 2	4
	GHS cat. 3	5
No label	GHS cat. 3	2
	GHS no cat.	9
Total		**20**

Table 4: Distribution of SIVS Phase 2 selected chemicals, according to the EU and GHS classification and labelling systems, and their database sources

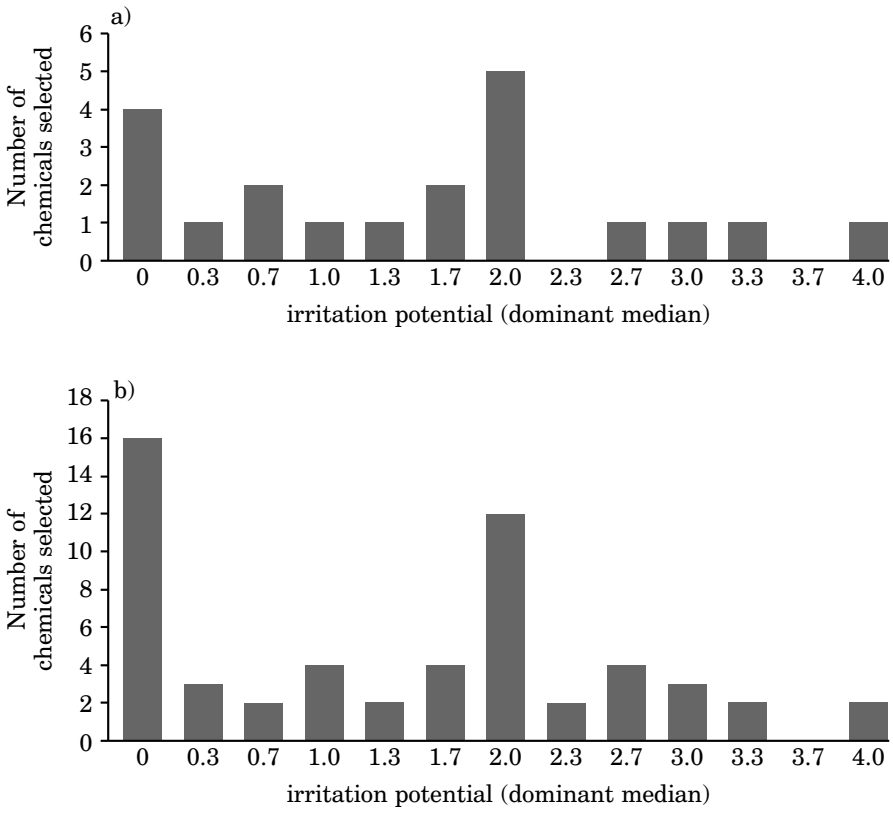
Source	R38 (skin irritants)		No label (non-irritants)		
	GHS cat. 2	GHS cat. 3	GHS cat. 3	GHS no cat.	Totals
NCD	7	9	3	14	33
ECETOC	5	2	2	10	19
TSCA+CIR	1	1	0	4	6
Totals	**25**		**33**		**58**

sible to select 60 chemicals for use in Phases 1 and 2 of the SIVS.

Phase 1 comprised 20 chemicals selected from the NCD (Table 3), with an almost-equal number of irritant (9 EU R38) versus non-irritant (11 EU no-label) substances, which corresponded well with the CSSC selection strategy shown in Figure 2, and with a balanced representation of the three GHS categories.

For Phase 2 of the SIVS, 60 chemicals were initially proposed by the CSSC, 35 selected from the NCD and 25 from the additional databases. Eighteen of these had been used in Phase 1 of the study. Unfortunately, due to confidentiality issues raised at the end of the study, the data obtained on two of the chemicals selected from the NCD (one R38 and one no-label) could not be disclosed. As a consequence, these two substances were disregarded, leaving 33 NCD chemicals and a total of 58 SIVS Phase 2 chemicals. The distributions of the Phase 2 chemicals according to the EU and GHS classification systems are shown in Table 4. Of the

Figure 3: The prevalence of dominant medians of the SIVS selected chemicals: a) Phase 1 (n = 20); b) Phase 2 (n = 58)

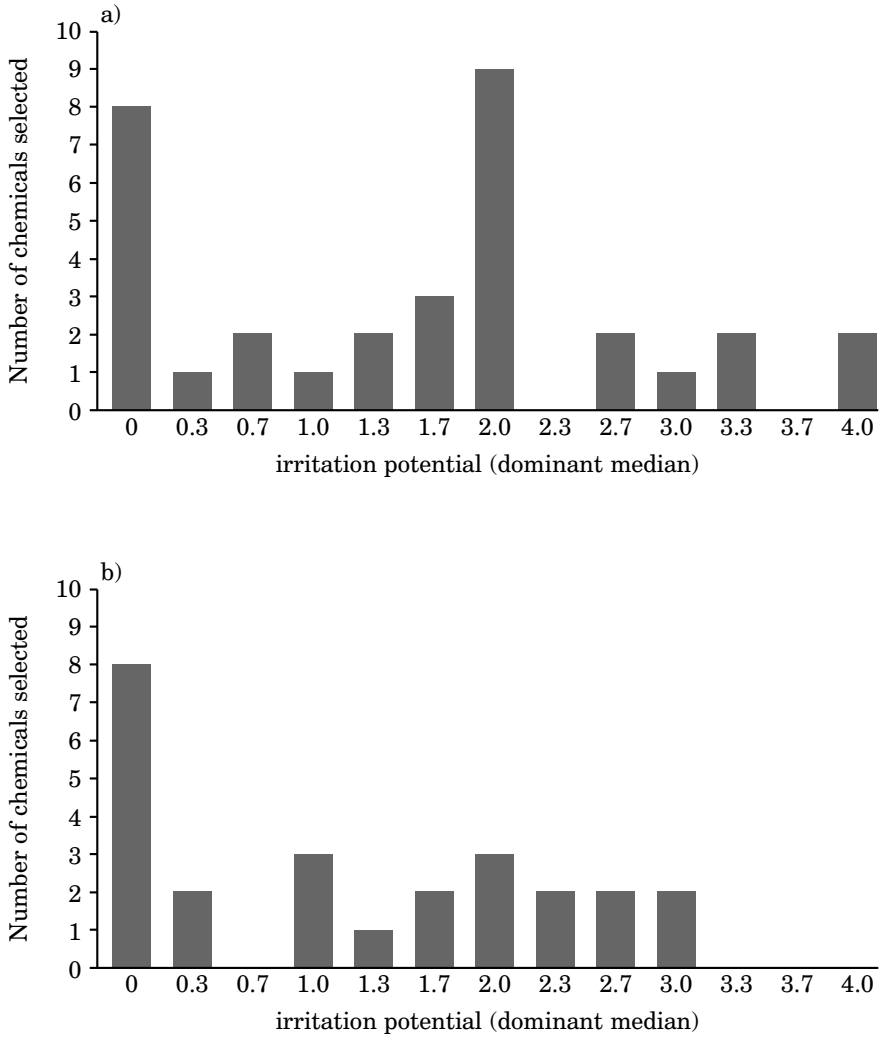


58 substances, 25 were irritants (labelled R38) and 33 were non-irritants (not labelled). This distribution corresponded with the CSSC strategy, i.e. to obtain 26 R38, and 34 no-label substances, prior to the subsequent exclusion of the two substances because of confidentiality issues.

With respect to the GHS classification system, SIVS Phase 2 chemicals comprised a balanced distribution of the three GHS categories, to allow for a *post hoc* evaluation. However, limitations were encountered in identifying GHS category 2 chemicals, as only 13 were found (instead of the 21 initially sought) among the 5100 chemicals screened. This may have been related to the low prevalence of GHS category 2 chemicals in the databases investigated. In compensation, 17 GHS category 3 chemicals were obtained (instead of the 10 initially foreseen), which is also in agreement with the findings of Hoffmann *et al.* (18), i.e. showing a higher prevalence of the *in vivo* responses observed close to the classification threshold of 2.

Figure 3 shows the range of irritancy covered by the SIVS Phase 1 and 2 chemicals, as characterised by the dominant median Draize test scores. A high number of substances with dominant median values of 0 were selected for the SIVS, in order to reflect the high prevalence observed for this category of compounds (18). In addition, Figure 4 shows the range of irritancy covered by the 33 'new' commercial chemicals registered in the NCD, and by the 25 'existing' chemicals recorded in the other databases. Both sets of chemicals had a similar distribution pattern, although the 'new' chemicals had a majority of scores in the

Figure 4: The prevalence of dominant medians of the SIVS Phase 2 selected chemicals: a) new commercial chemicals registered in the NCD (n = 33); b) existing chemicals selected from additional databases readily available from major commercial sources (n = 25)



severe range of irritancy and close to the classification threshold.

Information on the chemical identities, CAS numbers, physical states, purities and classification of the chemicals used in Phases 1 and 2 of the SIVS is shown in Table 5. In addition, complementary information on the commercial suppliers, product numbers and *in vivo* data for the 25 'existing' chemicals readily available from major commercial sources, is given in Table 6. The structural formulae of all the chemicals used in Phases 1 and 2 of the SIVS were also available (not shown). They presented a large variety of molecular structures and functional chemical groups. However, due to the fact that the notified chemicals registered in the NCD frequently present complex multi-functional molecular structures, typical of molecular structures engineered for a particular application, definitive allocation to a generic chemical class was precluded. Nevertheless, the selected chemicals covered a wide range of effect and use categories, relevant to current industrial commerce in the EU. In addition, as shown in Table 7, the selected compounds presented a wide range of physico–chemical properties, such as MW, log K_{ow}, water solubility, vapour pressure, melting point and boiling point.

Selected chemicals and the laboratory-related coding were organised by ECVAM and transferred to RCC–CCR (Research and Consulting Company–Cytotest Cell Research GmbH, Rossdorf, Germany), which then distributed the coded test samples to the participating laboratories.

6. Discussion

The ECVAM SIVS was the first formal validation study to make use of the ECB's NCD as the main source for the selection of test chemicals. This exercise proved to be successful in obtaining notified chemicals supported by systematic and reliable information. Data selected from the NCD were obtained from standard assays, conducted according to Annex V of *Directive 67/548/EEC* for regulating the marketing of industrial chemicals (1). Moreover, the information available from the NCD was generally comprehensive, and subject to quality compliance by the Competent Authorities of the EU Member States. However, notified chemicals are typically marketed for client-oriented industrial applications, so commercially-sensitive data are regarded as confidential. For this reason, the supporting data for two substances (one R38 and one no-label) could not be disclosed, so *in vitro* data for these chemicals could not be used in the analysis of the outcome of the SIVS. The use of the NCD therefore involved some limitations relating to company contact, material availability, and release of proprietary information. In contrast, 'existing' chemicals registered in other databases were readily commercially available from major manufacturing and/or distribution sources. However, the available information on their physico–chemical properties was not always retrievable in a standardised format, so there could be uncertainties with regard to their quality.

The sample size for the validation study was justified on the basis of statistical considerations. Although the application of the selection criteria, combined with practical constraints, limited the choice of candidate chemicals, the representativeness of the resulting sample population for the SIVS was considered not to have been compromised, as the introduction of potential biases during the selection process was avoided. The sample size for the validation study was justified on the basis of statistical considerations.

With such an approach, the results from the validation study could be interpreted objectively on a statistical basis. Adopting a rational statistical approach took into account the simple predictive capacity assessment underlying the study, expressed in terms of the binary result, specificity and sensitivity. In addition, the sample size calculation ensured that the specificity and sensitivity for a given power were significantly better than chance, i.e. larger than 50%. The sample sizes required would increase substantially, if the outcome included ordinal degree (i.e. more than two groups) or if the requirements of predictive capacity were more restrictive (e.g. to show that sensitivity and specificity are significantly larger than 60%, while maintaining the power). Then the prospect of a statistically-required number of chemicals which met pragmatic selection criteria would be unattainable. The discrepancy between the statistical ideal for chemical selection and the practical availability of material samples (and resources) in validation studies is rarely acknowledged, although it is well known. Such a dilemma might be an issue for consideration under the emerging concept of evidence-based toxicology (20, 21).

In conclusion, of the approximately 5100 chemicals screened, very few were found to fulfil the CSSC's pre-determined selection criteria for ensuring the quality of *in vivo* data and the practicability of testing. In particular, limitations were encountered in identifying GHS category 2 chemicals, most probably due to the low prevalence of such category of compounds (18). However, a total of 60 chemicals were finally selected and proposed for testing in Phases 1 and 2 of the SIVS. The distribution of chemicals selected by the CSSC: i) represented statistically justified sample sizes for distinguishing R38 from no-label chemicals; ii) provided a balanced representation of the three GHS categories, to allow for

Table 5: The identities of SIVS Phases 1 and 2 chemicals, with CAS number, physical state, purity, and EU and GHS classification and labelling based on *in vivo* Draize test scores

Chemical identification	CAS Number	Physical state	Typical purity (%)	Source	EU classification	GHS classification
(E,E)-3,7,11-trimethyldodeca-1,4,6,10-tetraen-3-ol	125474-34-2	L	95	NCD	R38	Cat. 2
[2-(cyclopentyloxy)ethyl]benzene (cyclopentyl 2-phenylethyl ether)	238088-70-5	L	98	NCD	R38	Cat. 2
benzenethiol, 5-(1,1-dimethylethyl)-2-methyl	7340-90-1	L	94	NCD	R38	Cat. 2
1-methyl-3-phenyl-1-piperazine	5271-27-2	S	99	NCD	R38	Cat. 2
cyclamen aldehyde	103-95-7	L	> 98[a]	ECETOC	R38	Cat. 2
1-decanol	112-30-1	L	98.8[a]	ECETOC	R38	Cat. 2
2-chloromethyl-3,5-dimethyl-4-methoxypyridine hydrochloride	86604-75-3	S	98.5	NCD	R38	Cat. 2
(+/–) *trans*-3,3-dimethyl-5-(2,2,3-trimethyl-cyclopent-3-en-1-yl)-pent-4-en-2-ol	107898-54-4	L	98.4	NCD[b]	R38	Cat. 2
1-bromohexane	111-25-1	L	>98.5[a]	ECETOC	R38	Cat. 2
α-terpineol	98-55-5	L	98.4[a]	ECETOC	R38	Cat. 2
di-n-propyl disulphide	629-19-6	L	99.2[a]	ECETOC	R38	Cat. 2
butyl methacrylate	97-88-1	L	>99[a]	TSCA	R38	Cat. 2
2-isopropyl-2-isobutyl-1,3-dimethoxypropane	129228-21-3	L	97.6	NCD	R38	Cat. 2
1-[4-(2-dimethylaminoethoxy)phenyl]-2-phenylbutan-1-one	68047-07-4	S	99	NCD	R38	Cat. 3
Mixture of isomers: 1-(2-isopropylphenyl)-1-phenylethane (CAS# 191044-60-7) 1-(3-isopropylphenyl)-1-phenylethane (CAS# 191044-59-4) 1-(4-isopropylphenyl)-1-phenylethane (CAS# 2320-06-1)	52783-21-8	L	96	NCD	R38	Cat. 3
Mixture of: 2-methyl-4-(2´,2´,3´-trimethyl-3´-cyclopenten-1´-yl)-4-penten-1-ol 56% (1´R,2R) & 40%(1´R,2S) isomer	014864-90-6	L	96	NCD	R38	Cat. 3
hexyl salicylate	6259-76-3	L	> 98[a]	ECETOC	R38	Cat. 3
terpinyl acetate	80-26-2	L	≥ 95[a]	ECETOC	R38	Cat. 3
tri-isobutyl phosphate	126-71-6	L	99.7[a]	TSCA	R38	Cat. 3
isostearic acid monoisopropanolamide	152848-22-1	L	95	NCD	R38	Cat. 3

[a]*information from ECETOC, TSCA or CIR*
[b]*pre-1995*
[c]*chemicals tested in Phase 1, but not in Phase 2 due to short shelf-lives*

Table 5: continued

Chemical identification	CAS Number	Physical state	Typical purity (%)	Source	EU classification	GHS classification
Mixture of isomers: ethyl exo-tricyclo[5.2.1.0(2,6)]decane-endo-2-carboxylate ethyl endo-tricyclo[5.2.1.0(2,6)]decane-exo-2-carboxylate	80657-64-3	L	99.6	NCD[b]	R38	Cat. 3
4-methyl-8-methylenetricyclo[3.3.1.1(3,7)]decan-2-ol	122760-84-3	S	99.4	NCD[b]	R38	Cat. 3
4-methyl-8-methylenetricyclo[3.3.1.1(3,7)]dec-2-yl acetate	122760-85-4	L	98.1	NCD[b]	R38	Cat. 3
bis[(1-methylimidazol)-(2-ethyl-hexanoate)], zinc complex	not allocated	L	99.6	NCD[b]	R38	Cat. 3
Mixture of isomers: 1-(1,1-dimethylpropyl)-4-ethoxy-*cis*-cyclohexane; 1-(1,1-dimethylpropyl)-4-ethoxy-*trans*-cyclohexane	181258-87-7 (*cis*) 181258-89-9 (*trans*)	L	99	NCD	R38	Cat. 3
diisononyl cyclohexane-1,2-dicarboxylate[c]	166412-78-8	L	99	NCD	R38	Cat. 3
2-phenylhexanenitrile	3508-98-3	L	99.5	NCD	Not classified	Cat. 3
allyl heptanoate	142-19-8	L	98.1[a]	ECETOC	Not classified	Cat. 3
heptyl butyrate	5870-93-9	L	> 95[a]	ECETOC	Not classified	Cat. 3
2-methyl-3-[(1,7,7-trimethylbicyclo[2.2.1]hept-2-yl)oxy]-1-propanol, bornyl isomer	128119-70-0	L	96	NCD	Not classified	Cat. 3
Mixture of: 5-exo-decylbicyclo[2.2.1]hept-2-ene; 5-endo-decylbicyclo[2.2.1]hept-2-ene	22094-85-5	L	99.6	NCD	Not classified	Cat. 3
2,6-dimethyl-4-nitrobenzeneamine	16947-63-0	S	99.5	NCD	Not classified	No category
2S-(2-furyl)-5R-hydroxy-4R-(1R,2-dihydroxy)ethyl-6S-hydroxymethyl-1,3-dioxane	7089-59-0	S	99.5	NCD	Not classified	No category
Mixture of isomers: 1-(spiro[4.5]dec-7-en-7-yl)pent-4-en-1-one (CAS# 224031-70-3); 1-(spiro[4.5]dec-6-en-7-yl)pent-4-en-1-one (CAS# 224031-71-4)	224031-70-3	L	96	NCD	Not classified	No category
propyl (2S)-2-(1,1-dimethylpropoxy)-propanoate	0319002-92-1	L	99.6	NCD	Not classified	No category
ethyl *cis*-4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine-1-carboxylate	67914-69-6	S	97.7	NCD	Not classified	No category
disodium 2,2´-(1,4-phenylene)bis-(1H-benzimidazole-4,6-disulphonic acid or monosulphonic acid, monosulphonate or disulphonate	180898-37-7	S	97.1	NCD	Not classified	No category
cyclohexadecanone	2550-52-9	S	99.2	NCD	Not classified	No category

[a]*information from ECETOC, TSCA or CIR*
[b]*pre-1995*
[c]*chemicals tested in Phase 1, but not in Phase 2 due to short shelf-lives*

Table 5: continued

Chemical identification	CAS Number	Physical state	Typical purity (%)	Source	EU classification	GHS classification
3-chloro-4-fluoronitrobenzene	350-30-1	S	98[a]	ECETOC	Not classified	No category
phenylethylalcohol	60-12-8	L	99.6[a]	ECETOC	Not classified	No category
allyl phenoxyacetate	7493-74-5	L	100[a]	ECETOC	Not classified	No category
4-methylthio-benzaldehyde	3446-89-7	L	98.2[a]	ECETOC	Not classified	No category
silane A-1430	2530-87-2	L	99.7[a]	TSCA	Not classified	No category
triethylene glycol	112-27-6	L	99.8[a]	TSCA	Not classified	No category
Mixture of: diethyl *cis*-1,4-cyclohexanedicarboxylate; diethyl *trans*-1,4-cyclohexanedicarboxylate	0072903-27-6	L	99	NCD	Not classified	No category
2-ethylhexyl 4-aminobenzoate	26218-04-2	S	99	NCD	Not classified	No category
capryl-isostearate	209802-43-7	L	99	NCD	Not classified	No category
methyl stearate	112-61-8	S	99[a]	ECETOC	Not classified	No category
3-diethylaminopropionitrile	5351-04-2	L	99.8[a]	ECETOC	Not classified	No category
1-bromo-4-chlorobutane	6940-78-9	L	98[a]	ECETOC	Not classified	No category
diethyl phthalate	84-66-2	L	99.7[a]	ECETOC	Not classified	No category
di-propylene glycol	25265-71-8	L	99[a]	ECETOC	Not classified	No category
isopropanol	67-63-0	L	100[a]	ECETOC	Not classified	No category
dipropylene glycol monobutyl ether	29911-28-2	L	> 99[a]	CIR	Not classified	No category
naphthalene acetic acid	86-87-3	S	96[a]	TSCA	Not classified	No category
2,5-dimethyl-4-oxo-4,5-dihydrofuran-3-yl acetate	4166-20-5	L	98	NCD	Not classified	No category
3-mercaptohexanol	51755-83-0	L	98.5	NCD	Not classified	No category
3,4-dimethyl-1H-pyrazole	2820-37-3	S	99	NCD	Not classified	No category
2-(formylamino)-3-thiophenecarboxylic acid	43028-69-9	S	96	NCD	Not classified	No category
Mixture of: (E)-oxacyclohexadec-12-en-2-one; (E)-oxacyclohexadec-13-en-2-one; a) (Z)-oxacyclohexadec-12-en-2-one and b) (Z)-oxacyclohexadec-13-en-2-one[c]	not allocated	L	96	NCD	Not classified	No category

[a] *information from ECETOC, TSCA or CIR*
[b] *pre-1995*
[c] *chemicals tested in Phase 1, but not in Phase 2 due to short shelf-lives*

Table 6: Commercial suppliers, product numbers and *in vivo* data for the 'existing' chemicals, readily available from major commercial sources[a]

Chemical name	Commercial supplier	Product number	No. of experiments	No. of rabbits	Rabbit erythema scores (average of scores after 24, 48, 72h)														Erythema median	Dominant endpoint	Dominant median	EU classification	GHS classification
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15					
a) Rabbit erythema scores																								
butyl methacrylate	Sigma	235865	1	6	3.0	3.0	2.7	3.0	3.0	3.0										3.0	E	3.0	R38	Cat. 2
di-n-propyl disulphide	Sigma	149225	1	3	1.7	3.0	3.0													3.0	E	3.0	R38	Cat. 2
1-bromohexane	Sigma	B68240	1	3	2.7	2.0	2.7													2.7	E	2.7	R38	Cat. 2
α-terpineol	Sigma	T3407	3	11	1.7	2.0	2.3	2.0	2.7	2.0	1.7	2.0	2.0	1.7	2.0					2.0	O	2.0	R38	Cat. 2
1-decanol	Sigma	150584	1	4	2.3	2.3	2.3	1.7												2.3	E	2.3	R38	Cat. 2
cyclamen aldehyde	Givaudan	1534001	4	15	2.7	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.7	2.0	2.0	2.0	2.0	2.0	O	2.0	R38	Cat. 2
hexyl salicylate	Sigma	84280	4	15	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	B	2.0	R38	Cat. 3
terpinyl acetate	Sigma	W304700	3	11	1.7	2.0	2.0	2.0	2.0	2.0	2.0	2.0	1.7	2.0	1.3					2.0	B	2.0	R38	Cat. 3
tri-isobutyl phosphate	Sigma	92035	1	6	2.0	2.3	1.7	2.0	2.3	2.0										2.0	E	2.0	R38	Cat. 3
allyl heptanoate	Sigma	W203106	1	4	1.3	2.0	1.7	2.0												1.7	E	1.7	NC	Cat. 3
heptyl butyrate	Sigma	W254908	1	4	1.7	2.0	0.7	2.0												1.7	E	1.7	NC	Cat. 3
3-chloro-4-fluoronitrobenzene	Sigma	233234	1	6	1.7	1.0	1.0	1.7	1.7	1.0	1.7									1.3	E	1.3	NC	No cat.
4-methylthio-benzaldehyde	Sigma	222771	1	3	1.0	1.3	0.3													1.0	E	1.0	NC	No cat.
methyl stearate	Sigma	335185	1	3	1.0	2.3	1.0													1.0	E	1.0	NC	No cat.
phenylethylalcohol	Sigma	77861	2	7	1.3	1.3	2.0	1.0	1.0	0.7	0.3									1.0	E	1.0	NC	No cat.
isopropanol	Sigma	270490	1	3	1.7	0.3	0.3													0.3	E	0.3	NC	No cat.
allyl phenoxyacetate	Sigma	W203807	1	4	0.3	0.7	0.3	0.3												0.3	E	0.3	NC	No cat.
1-bromo-4-chlorobutane	Sigma	16662	1	3	0.0	0.0	0.0													0.0	B	0.0	NC	No cat.
3-diethylaminopropionitrile	Acros Organics	123191000	1	3	0.0	0.0	0.0													0.0	B	0.0	NC	No cat.
diethyl phthalate	Sigma	524972	2	7	0.7	0.0	0.0	0.0	0.0	0.0	0.0									0.0	E	0.0	NC	No cat.
di-propylene glycol	Sigma	D215554	2	7	1.0	0.0	0.0	0.0	0.0	0.0	0.0									0.0	E	0.0	NC	No cat.
dipropylene glycol monobutyl ether	Sigma	388130	1	6	0.3	0.0	0.0	0.0	0.0	0.0										0.0	E	0.0	NC	No cat.
naphthalene acetic acid	Sigma	N0640	1	6	0.0	0.0	0.0	0.0	0.0	0.0										0.0	B	0.0	NC	No cat.
silane A-1430	Sigma	440183	1	6	0.0	0.0	0.0	0.0	0.0	0.0										0.0	B	0.0	NC	No cat.
triethylene glycol	Sigma	95126	1	6	0.0	0.0	0.0	0.0	0.0	0.0										0.0	B	0.0	NC	No cat.

[a]*Skin irritant chemicals were classified on the basis of severity of effects, and not based on persistence. For the non-classified chemicals, all skin irritation effects reversed before 14 days.*

NC = not classified; No cat. = no category; E = erythema; O = oedema; B = both.

Table 6: continued

Chemical name	Commercial supplier	Product number	No. of experiments	No. of rabbits	Rabbit oedema scores (average of scores after 24, 48, 72h)														Oedema median	Dominant endpoint	Dominant median	EU classification	GHS classification
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15					
b) Rabbit oedema scores																								
butyl methacrylate	Sigma	235865	1	6	2.7	3.7	2.3	2.7	2.7	4.0										2.7	E	3.0	R38	Cat. 2
di-n-propyl disulphide	Sigma	149225	1	3	0.0	0.0	0.0													0.0	E	3.0	R38	Cat. 2
1-bromohexane	Sigma	B68240	1	3	0.0	2.7	2.0													2.0	E	2.7	R38	Cat. 2
α-terpineol	Sigma	T3407	3	11	2.0	2.3	3.0	3.0	3.0	2.7	1.7	2.7	2.0	0.7	3.0					2.7	O	2.0	R38	Cat. 2
1-decanol	Sigma	150584	1	4	2.0	1.0	1.0	1.0												1.0	E	2.3	R38	Cat. 2
cyclamen aldehyde	Givaudan	1534001	4	15	3.0	3.0	2.7	2.3	2.7	2.0	1.7	2.7	2.7	2.3	3.0	1.3	1.0	2.0	1.3	2.3	O	2.0	R38	Cat. 2
hexyl salicylate	Sigma	84280	4	15	1.0	1.3	2.0	0.7	2.0	2.0	2.0	2.7	1.7	2.0	2.7	1.3	2.0	1.0	1.0	2.0	B	2.0	R38	Cat. 3
terpinyl acetate	Sigma	W304700	3	11	1.0	2.0	2.0	3.0	2.0	2.3	2.0	2.0	1.0	0.7	0.3					2.0	B	2.0	R38	Cat. 3
tri-isobutyl phosphate	Sigma	92035	1	6	1.0	1.3	0.7	1.3	1.0	0.0										1.0	E	2.0	R38	Cat. 3
allyl heptanoate	Sigma	W203106	1	4	0.3	0.7	0.7	0.7												0.7	E	1.7	NC	Cat. 3
heptyl butyrate	Sigma	W254908	1	4	0.0	0.3	0.0	0.7												0.3	E	1.7	NC	Cat. 3
3-chloro-4-fluoronitrobenzene	Sigma	233234	1	6	0.7	0.0	0.0	0.7	0.7	0.0										0.3	E	1.3	NC	No cat.
4-methylthio benzaldehyde	Sigma	222771	1	3	0.0	0.0	0.0													0.0	E	1.0	NC	No cat.
methyl stearate	Sigma	335185	1	3	0.0	2.0	0.0													0.0	E	1.0	NC	No cat.
phenylethylalcohol	Sigma	77861	2	7	0.0	1.0	1.0	0.0	0.7	0.0	0.0									0.0	E	1.0	NC	No cat.
isopropanol	Sigma	270490	1	3	0.0	0.0	0.0													0.0	E	0.3	NC	No cat.
allyl phenoxyacetate	Sigma	W203807	1	4	0.3	0.0	0.0	0.3												0.0	E	0.3	NC	No cat.
1-bromo-4-chlorobutane	Sigma	16662	1	3	0.0	0.0	0.0													0.0	B	0.0	NC	No cat.
3-diethylaminopropionitrile	Acros Organics	123191000	1	3	0.0	0.0	0.0													0.0	B	0.0	NC	No cat.
diethyl phthalate	Sigma	524972	2	7	0.0	0.0	0.0	0.0	0.0	0.0	0.0									0.0	E	0.0	NC	No cat.
di-propylene glycol	Sigma	D215554	2	7	0.0	0.0	0.0	0.0	0.0	0.0	0.0									0.0	E	0.0	NC	No cat.
dipropylene glycol monobutyl ether	Sigma	388130	1	6	0.0	0.0	0.0	0.0	0.0	0.0										0.0	E	0.0	NC	No cat.
naphthalene acetic acid	Sigma	N0640	1	6	0.0	0.0	0.0	0.0	0.0	0.0										0.0	B	0.0	NC	No cat.
silane A-1430	Sigma	440183	1	6	0.0	0.0	0.0	0.0	0.0	0.0										0.0	B	0.0	NC	No cat.
triethylene glycol	Sigma	95126	1	6	0.0	0.0	0.0	0.0	0.0	0.0										0.0	B	0.0	NC	No cat.

[a]*Skin irritant chemicals were classified on the basis of severity of effects, and not based on persistence. For the non-classified chemicals, all skin irritation effects reversed before 14 days.*

NC = not classified; No cat. = no category; E = erythema; O = oedema; B = both.

Table 7: Range of physico–chemical properties covered by the chemicals selected for SIVS Phase 2

Physico–chemical property	Observed range
Molecular weight	60 to 674
Log K_{ow}[a]	–3.4 to 11.5
Water solubility[b]	10^{-3} to 10^{6} mg/l
Vapour pressure[b]	10^{-6} to 6×10^{3} Pa
Melting point	–100 to 360°C
Boiling point	82 to 555°C

[a]*Measured at temperatures ranging from 19 to 30°C.*
[b]*Measured at temperatures ranging from 19 to 25°C.*

the *post hoc* evaluation of the performance of the assays for that classification system; and iii) represented a high proportion of substances with dominant median values of 0, similar to the prevalence distribution found by Hoffmann *et al.* (18). In addition, the selected compounds presented a variety of molecular structures, functional chemical groups, and effect and use categories, as well as a wide range of physico–chemical properties. Therefore, the participating test laboratories were presented with a challenging set of chemicals, relevant to the current world of industrial commerce, and appropriate for the evaluation of the capacity of the EpiDerm, EPISKIN and the SIFT alternative methods to reliably identify skin irritant and non-irritant chemicals.

7. Acknowledgements

The CSSC wishes to acknowledge the support given by Dr Dave Allen, Dr Raymond Tice and Dr William Stokes (NICEATM), who provided a list of candidate substances of potential value to the SIVS. In addition, the CSSC gratefully acknowledges the contributions made by the 25 companies that collaborated with the SIVS by providing test samples and disclosing the sensitive data used in the study.

Received 15.11.07; accepted for publication 21.11.07.

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