chance correlation. The Linear Learning Machine (LLM) and Iterative Least Squares (ILS) linear discriminant models are suitable for predicting new samples because of their low probability of overfitting.

More extensive researches, e.g. further studies of large amounts of experimental data, revisions of feature selection according to certain hypotheses of parameters, the addition of new parameters and selecting suitable discriminant function for respiratory sensitization, are needed to investigate respiratory QSAR system.

Although QSAR systems are still being developed and have yet to become sufficiently powerful, the use of *in silico* methods has been proposed to make predictions of respiratory sensitization in the first stage of a decision-tree testing strategy for respiratory sensitization (Seed *et al.*, 2008). In this study, classification rates were 99.53% to 100%. Prediction rates (CV) were 95.33% to 97.2%. This QSAR system is thought to be applicable to initial prediction of the respiratory sensitizing ability of untested chemicals.

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Corresponding author:
Kazuhiro Sato, Ph.D.,
Department of Environmental Health,
School of Medicine, University of Fukui,
Shimoaizuki 23-3, Matsuoka, Eiheiji-cho,
Yoshida-gun, Fukui 910-1193, Japan
Tel.:+81-776-61-8338
Fax:+81-776-61-8107
E-mail: satokazu@u-fukui.ac.jp

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Validation study of the new criteria for sensitizer using German sensitizers of Deutschen Forschungsgemeinschaft (DFG)

Kazuhiro Sato*, Research group of classification and listing of sensitizer.
Department of Environmental Health, School of Medicine, University of Fukui, Eiheiji-cho, Fukui 910-1193, Japan
Tel: +81-776-61-8338, Fax: +81-776-61-8107, e-mali: satokazu@u-fukui.ac.jp
*Corresponding author

1 Table

Key words: [1]criteria for sensitizer, [2]animal studies, [3]globally harmonized system of classification and labeling of chemicals (GHS)

ABSTRACT

The globally harmonized system of classification and labeling of chemicals (GHS) was recommended by United Nations (UN) and became available in 2008 all over the world. The classification criteria for skin and airway sensitizers in GHS include evidences from animal studies, for example, OECD Guideline 406 (guinea pig maximization test, GPMT and Buhler guinea pig test) and Guideline 429 (local lymph node assay, LLNA). According to Deutschen Forschungsgemeinschaft (DFG) in Germany and European Chemical Bureau (ECB), the criteria for sensitizers also include evidences from validated animal studies. At present recognized and validated animal models for the testing of respiratory hypersensitivity are not available. In Japan, the criteria from the Japan Society for Occupational Health (JSOH) for sensitizers do not include evidences from animal studies. We revised the criteria for sensitizers of JSOH and adopted evidences of animal studies. We organized the research group for sensitizer in 2005 and reviewed the criteria of Germany, EU, GHS and so on (19 experts). The meetings were held twelve times and made the revised criteria for sensitizer which adopted animal studies. We tried to validate the criteria using 28 German sensitizers of DFG, which were not listed in JSOH.

We could correctly classify 24 sensitizers by our revised criteria, however, four sensitizers could not be classified at first. Therefore, we visited the secretariat of the committee of DFG in Freising, Germany to investigate the evidenced papers of these four sensitizers in October, 2008. We could find out the evidenced papers of two, however, two sensitizers could not be classified at last. We could correctly classify 26 out of 28 sensitizers. We concluded that our revised criteria were appropriate and that this validation study was successful.

There are over 30 million chemicals in the world. Many countries address classification and labeling for at least some chemicals in their countries. In the area of trade, the need to comply with multiple regulations regarding hazard classification and labeling is costly and time-consuming (confusing). These differences impact both protection and trade. In the area of protection, users in countries that don't have specific requirements may see different label warnings or data sheet information for the same chemical.

In July 2003, UN recommended and published globally harmonized system of classification and labeling of chemicals (GHS). GHS became available in 2008 all over the world (1). The classification criteria for skin sensitizers in GHS adopt evidences of validated animal studies, e.g. OECD guideline 406 (guinea pig maximization test, GPMT and Buhler test) and Guideline 429 (Local Lymph Node Assay, LLNA). The criteria for skin sensitizers of DFG in Germany (2) and European Chemical Bureau (ECB) (3) in European Union (EU) also adopt evidences of validated animal studies. There are some qualitative and quantitative relationships between the results of validated animal studies and human data (4). However, in Japan, Criteria for sensitizer of Japan Society for Occupational Health (JSOH) for sensitizers do not adopt evidences of animal studies (5) and are revised (6).

The definition of respiratory sensitization is that a respiratory sensitizer is a substance that will lead to hypersensitivity of the airways following inhalation of the substance and that at present, recognized and validated animal models for the testing of respiratory hypersensitivity are not available. Under certain circumstances, data from animal studies may provide valuable information in a weight of evidence assessment. The definition of skin sensitizer is that a skin sensitization is a substance that will lead to an allergic response following skin contact and that at present, evidence from animal studies, e.g. guinea pig maximization test (GPMT), Buhler test in OECD406, mouse local lymph node assay (LLNA) in OECD429 is usually more reliable than evidence from human exposure in reproducibility and handling (1,2). However, in cases where evidence is available from both sources, and there is conflict between the results, the quality and reliability of the evidence from both sources must be assessed in order to resolve the question of classification on a case-by-case basis (1).

JSOH Criteria of sensitizer has adopted only human evidences and has not adopted animal evidences till now (5). Therefore, we organized the research group of classification and listing of sensitizers, reviewed the criteria for sensitizer in German DFG (2), German dermatologists group (7), European Chemical Bureau (ECB) (3) and American Conference of Governmental Industrial Hygienists (ACGIH) (8) and revised the criteria for sensitizer in JSOH (5,6).

The criteria for sensitizer in German DFG (2) adopts validated animal studies and describe that at present, the sensitization potential of a substance can be best be estimated in animal studies in skin sensitization. Sensitizers in German DFG (2) are 234. German dermatologists group (7) also adopts appropriate animal studies in the criteria for skin sensitization and lists 244 skin sensitizers. The criteria of ECB (3) for sensitizer adopt appropriate animal studies and ECB lists R42 (respiratory sensitizer): 29, R42/43 (respiratory and skin sensitizer): 86, R43: 910 (skin sensitizer). In ACGIH, the designation "SEN" in the "Notation" column refers to the potential for an agent to produce sensitization, as confirmed by human or animal data and 26 sensitizers are listed (8). The list of sensitizers in JSOH (5) includes 18 airway and 32 skin sensitizers. We should expand the list of sensitizers in JSOH (5).

MATERIALS AND METHODS

Our research group held meetings twelve times to revise the criteria for sensitizer and reclassify the sensitizers of JSOH (5) from August 2005 to February 2010. Our new criteria for sensitizer are as follows (6);

Definition of sensitizers

Respiratory sensitizers are substances which can induce respiratory sensitization* by inhalation. Skin sensitizers are substances which can induce specific allergic skin sensitization by skin contact.

*rhinitis, asthma, hypersensitivity pneumonitis, eosinophilic pneumonia and so on.

Classification of sensitizers

Occupational sensitizers are recommended for respiratory tract and skin. The sensitizers are

classified into Group 1 substances which induce allergic reactions in humans, Group 2 substances which probably induce allergic reactions in humans and Group 3 substances which possibly induce allergic reactions in humans.

Occupational exposure limits

Recommendation of occupational exposure limits for the occupational sensitizers does not necessarily consider either prevention of induction or elicitation. Respiratory sensitization might be severe to human health.

Respiratory sensitizers

Group 1

There is a clear association between respiratory symptoms and occupational exposure. Case reports of positive inhalation challenge tests, of positive serological studies or positive prick tests are reported in at least two different research organizations.

and

There is at least one epidemiological study which indicate a clear association between respiratory symptoms and occupational exposure.

Group 2

There is a clear association between respiratory symptoms and occupational exposure. Case reports of positive inhalation challenge tests, positive serological studies or positive prick tests are reported in at least two different research organizations.

However, there isn't an epidemiological study.

Group 3

(1) Positive animal tests which fulfill all conditions as below are reported in at least two different research organizations.

(i) induction and elicitation are performed by inhalation, nasal or bronchial administration.

(ii) detections of elicitation are performed by either bronchial alveolar lavage fluid (BALF), cell fractionation or histopathological studies. One of respiratory function studies, detections of antibodies or analyses of cytokines are performed.

(iii) both only induction group and only elicitation group are set up as negative control.

(iv) clear positive control. is integrated.

or

(2) Positive animal test which fulfills all conditions as stated above is reported in only one research organization. Positive appropriate animal test which does not fulfill all conditions is also reported in other research organization.

*Other conditions than a set of the all stated above ((i)~(iv)) might also provide an evidence for a sensitizer to animals.

Skin sensitizers

Group 1

Case reports which show a clear association between skin symptoms and patch tests are reported in at least two different research organizations.

and

An epidemiological study which clearly indicate associations among occupational exposures, symptoms of contact dermatitis and patch tests is also reported in at least one research organization. Patch tests should be appropriately performed with controls.

Group 2

Case reports which show a clear association between skin symptoms and patch tests are reported in at least two different research organizations.

However, there isn't an epidemiological study.

Group 3

Positive appropriate animal tests; guinea pig maximization test (GPMT) and the Buehler guinea pig test (OECD Guideline 406) or Local Lymph Node Assay (LLNA) (OECD 429) are reported in at least one

research organization.

Response of 30% or or more is considered as positive in GPMT. Response of 15% or more is considered as positive in Buehler test. Stimulation Index (SI) should be 3 or more in LLNA. Other animal tests which are validated would be taken into account.

Our research group reviewed the criteria in German DFG (2), ECB (3) and German dermatologist group (7) and ACGIH (8), revised the criteria of JSOH (5) and reclassified 28 German DFG (2) which were not listed in JSOH (5) using our new criteria for sensitizer (6).

RESULTS

We could reclassify 24 sensitizers correctly (Table I). However, butane oxime, m-chloroaniline, 1,5-diaminonaphthalene and 3,4-dichloroaniline could not be classified by our new criteria.

Therefore two members of our research group visited the secretariat of the commission for the investigation of health hazards of chemical compounds in the work area in Freising, Germany. We got the evidenced papers of these four substances. We found out new papers in butane oxime (9,10,11) and m-chloroaniline (12,13). We could reclassify these two substances. However, the evidenced papers of 1,5-diaminonaphthalene and 3,4-dichloroaniline were chemical company secret data. We could not classify these two substances at last.

DISCUSSION

To adopt the evidences of human and animal studies is the trend of the criteria for sensitizer in the world (1,2,3,5,7). Our research group made the new criteria for sensitizer (6) which adopted the evidences of animal studies and performed the validation study of the criteria using German DFG sensitizers (2).

The result of validation study was that 26 of 28 sensitizers could be classified by our new criteria. We concluded that this validation study was successful, this criteria were practical and that number of sensitizers should be expanded.

Furthermore, under the new European Union (EU) Registration, Evaluation and Authorization of Chemicals (REACH) rules, all chemicals in the EU that are produced or imported in quantities of more than one ton per annum will need to be assessed as potential human and environmental hazards, for example, in terms of their carcinogenicity; human sensitivity to such chemicals will also need to be determined. REACH calls for increased use of hazard assessment alternatives such as *in vitro* methods and quantitative structure-activity relationships (QSARs). Since no *in vitro* replacement is currently available for skin sensitization, nor is expected to be ready in the near future, the use of QSAR approaches presents an attractive alternative (14). The legislative trend towards the abolition of the animal testing of cosmetic products in the seventh Amending Directive 2003/15/EC to Cosmetics Directive 76/768/EEC includes a demand for alternative evaluation procedures (15). We made QSAR models for skin and respiratory sensitizer using ADMEWORKS/ModelBuilder software (Fujitsu Kyushu Systems Limited, Japan) (16).

*members of the research group of classification and listing of sensitizer are as follows;

Chairman: Yukinori Kusaka (University of Fukui)

Advisory: Toshio Matsushita (University of Kagoshima)

Member: Kohji Aoyama (University of Kagoshima)
Atsushi Ueda (Kumamoto University)
Kohichi Harada (Kumamoto University)
Keiko Minamoto (Kumamoto University)
Takemi Otsuki (Kawasaki Medical School)
Kunihiko Yamashita (Daicel Chemical Industries)
Tatsuya Takeshita (Wakayama Medical University)
Eiji Shibata (Aichi Medical University)
Kazuhiro Sato (University of Fukui)
Tomohiro Umemura (University of Fukui)

Taro Tamura (University of Fukui)
Muneyuki Miyagawa (National Institute of Occupational Safety and Health)
Masaaki Kaniwa (National Institute of Health Sciences)
Kunio Dobashi (Gunma University)
Satomi Kameo (Gunma University)
Tetsuhito Fukushima (Fukushima Medical University)
Takahiko Yoshida (Asahikawa Medical University)

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Table I. Results of reclassifying German DFG sensitizers by our new criteria

Abietic acid(Sh)(514-10-3)	Skin 1
Acrylamide(Sh)(79-06-1)	Skin 2
2-Aminoethanol(Sh)(141-43-5)	Skin 2
Ammonium persulphate(Sah)(7727-54-0)	Airway 1, Skin 1
α-Amylase(Sa)(EC3.2.1.1)	Airway 1
α-Amylcinnamaldehyde(Sh)(122-40-7)	Skin 2
Aniline(Sh)(62-3-3)	Skin 1
Benomyl(Sh)(17804-35-2)	Skin 1
Benzylalcohol mono(poly)hemiformal(Sh)((14548-60-8)	Skin 2
Brolemain(Sa)(9001-00-7)	Airway 2
1,4-Butanediol diacrylate(Sh)(1070-70-8)	Skin 3
Bithionol(SP)(97-18-7)	Skin 2
Butane oxime(Sh)(96-29-7)	*Skin 3
n-Butyl glycyl ether(BGE)(Sh)(2426-08-6)	Skin 2
n-Butyl methacrylate(Sh)(97-88-1)	Skin 2
p-tert-Butyl phenol(Sh)(98-54-4)	Skin 2
Butynediol(Sh)(110-65-6)	Skin 2
Chloroacetamide-N-methylol(Sh)(2832-19-1)	Skin 2
m-Chloroaniline(Sh)(108-42-9)	*Skin 2
Dicyclohexylcarbodiimide(Sh)(538-75-0)	Skin 2
1-Chloro-2,4-dinitrobenzene(Sh)(97-00-7)	Skin 1
Cinnamaldeyde(Sh)(104-55-2)	Skin 2
Chlorothalonil(Sh)(1897-45-6))	Skin 1
Cinnamyl alcohol(Sh)(104-54-1)	Skin 2
4,4-Diaminodiphenylmethane(Sh)(101-77-9)	Skin 1
1,5-Diaminonaphthalene(Sh)(2243-62-1)	*Difficulty
1,2-Dibromo-2,4-dicyanobutane(Sh)(35691-65-7)	Skin 2
3,4-Dichloroaniline(Sh)(95-76-1)	*Difficulty

*Dificult to classify