報告書[1]によれば、近年「柔軟剤のにおい」に関する相談が増加する傾向にあり、2008年度以降の計187件の相談のうち、60%を超える115件が頭痛や吐き気などの体調不良を訴える内容であった。また、強い芳香のある柔軟剤あるいは微香タイプの柔軟剤を使用した場合、柔軟剤を使用しない場合について、室内空気中の総揮発性有機化合物 (TVOC) を調べた結果、柔軟剤を使用しない場合と微香タイプの柔軟剤を使用した場合ではTVOCがそれぞれ約20 μg/m^{3}上昇したのに対し、強い芳香のある柔軟剤を使用した場合のTVOCの増加は3～7倍 (約70～140 μg/m^3) に達することが報告されている。

ただし、TVOCの増加と柔軟剤のにおいによる健康被害を直接結びつけることは困難であり、香料成分によって引き起こされる可能性のある咳や頭痛、吐き気についてもそれらの発生機序が十分に明らかにされているわけではない。

そこで、本研究事業の初年度において、Formaldehydeや Acroleinなどのアルデヒド類や防腐剤パラベン、抗菌剤など多様な室内環境化学物質の生体内標的分子であり、これらの化学物質による気道刺激などにも関与するTRPA1 (Transient Receptor Potential Cation Channel, Subfamily A, Member 1) に着目し、柔軟剤の揮発性成分による活性化について検討を行った。

その結果、市販の高残香性衣料用柔軟仕上げ剤を対象として、揮発成分をディスク型MonoTrap DCC18 (ジーエルサイエンス) に吸着させ、そのMethanol抽出液を柔軟剤抽出液として、ヒト侵害刺激受容体TRPA1の活性化能を検討した結果、評価した20製品中18製品で用量依存なTRPA1の活性化が認められた。

そこで本研究では、欧州連合の化粧品指令でアレルギー物質としてラベル表示を義務付けられた香料成分を対象として、ヒトTRPV1及びTRPA1の安定発現細胞株を用いて、細胞内 Ca^{2+}濃度の増加を指標として対象化合物のイオンチャネルの活性化能を評価した。

B. 実験方法

B-1. TRPイオンチャネル発現細胞による気道刺激性の評価

ヒト後根神経節Total RNAよりRT-PCRによってTRPV1及びTRPA1 cDNAをpENTR/D-TOPO Vectorにクローニングし、Gateway LR反応によりpEF5/ERT/V5-DEST Vectorにサブクローニングした。得られたPlasmidをLipofectamine LTXをもちいてpOG44 Vectorとともに Flp-In 293細胞にCo-transfectionし、Hygromycin Bを含む選択培地中で培養を行って耐性細胞株を選択してヒトTRPV1及びTRPA1安定発現細胞株を樹立した (TRPV1/HEK293, TRPA1/ HEK293)。

樹立したヒトTRPV1及びTRPA1安定発現細胞を用いて細胞内 Ca^{2+}濃度の増加を指標として被検物質によるTRPV1及びTRPA1イオンチャネルの活性化を評価した。細胞内 Ca^{2+}濃度の測定にはFLIPR Calcium 6 Assay Kit (Molecular Devices,

Inc.) を用いた。 96-well plate に hTRPV1/Flp-In 293 又は TRPA1/ Flp-In 293 を 1well あたり 4×10^4 個播種した。24 時間培養後に、培地を除去し Calcium indicator (Calcium 6) を添加して 37° C で 1 時間インキュベーションしたのち、被検物質の DMSO 溶液を添加し、FlexStation 3 (Molecular Devices, Inc.) において、被検物質添加後の蛍光強度の経時的な変化を励起波長 485 nm、蛍光波長 525 nm の条件で測定した。活性化の程度は、相対蛍光強度 (Relative fluorescence units) の差、あるいはそれぞれのイオンチャネルの典型的な活性化物質である Capsaicin (TRPV1) 及び Cinnamic aldehyde (TRPA1) による活性化能に対する比率で示した。

評価に用いる化合物は、可能な限り高純度の試薬を入手した。評価の対象とした化合物を図 1 に示す。

B-4. 統計的解析手法

結果の解析は Prism 5.00 (GraphPad Software, San Diego, CA) を用いた。

C. 結果と考察

香料アレルゲンとして表示義務のある香料リストのうち植物エキス等を除いて今回評価可能であった 17 物質中 8 物質が濃度依存的に TRPA1 の活性化を引き起こすことが判明した(図 1)。なかでも、2-(4-tert-Butylbenzyl) propionaldehyde による TRPA1 の活性化の程度は陽性対象物質である Cinnamaldehyde に匹敵することが明らかとなった。ヒト TRPV1 については、本検討条件においては活性化を引き起こす物質は認められなかった。以上の結果は、これら香料アレルゲンが TRPA1 の活性化を介して気道過敏の亢進を引き起こす可能性を示唆しており、シックハウス症候群の発症メカニズムを明らかにする上でも極めて重要な情報であると考えられる。

D. 結論

欧州において香料アレルゲンとして表示義務のある香料リストのうち 18 物質中 9 物質が、濃度依存的に TRPA1 の活性化を引き起こすことを明らかにした。

E. 健康危険情報

なし

F. 研究発表

論文発表

なし

学会発表（予定）

1. 香川(田中) 聡子,大河原 晋, 埴岡伸光, 神野透人: 香料アレルゲンによるヒト侵害受容器 TRPA1 の活性化. 第 43 回日本毒性学会学術年会 (2016.6)

G. 知的財産権の出願・登録状況 (予定を含む)

特許取得

なし

実用新案登録

なし

H. 引用文献

1. 柔軟仕上げ剤のにおいに関する情報提供, 独立行政法人国民生活センター, 2013 年 9 月 19 日公表
http://www.kokusen.go.jp/news/data/n-20130919_1.html

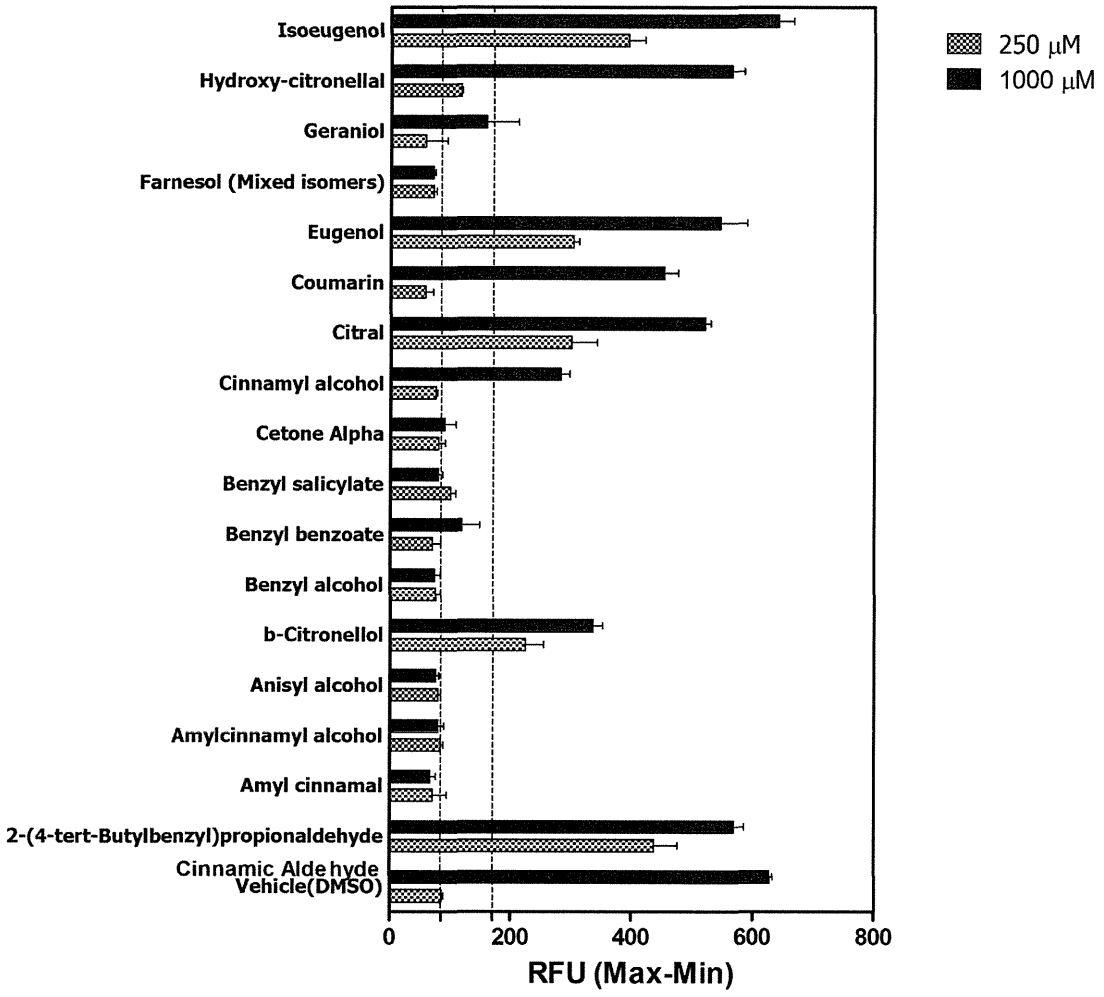

図 1　香料アレルゲンによるヒト TRPA1 イオンチャネルの活性化

III. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Kawakami T., Isama K., Ikarashi Y.	Particle size distribution of aerosols sprayed from household hand-pump sprays containing fluorine-based and silicone-based compounds.	Bull. Nalt. Inst. Health Sci.	133	37-41	2015
	.			-	
.	.				

IV. 研究成果の刊行物・別刷

Bull. Natl Inst. Health Sci., **133**, 37-41 (2015) **Technical Data**

Particle size distribution of aerosols sprayed from household hand-pump sprays containing fluorine-based and silicone-based compounds

Tsuyoshi Kawakami[#], Kazuo Isama, Yoshiaki Ikarashi

Japan has published safety guideline on waterproof aerosol sprays. Furthermore, the Aerosol Industry Association of Japan has adopted voluntary regulations on waterproof aerosol sprays. Aerosol particles of diameter less than 10 μm are considered as "fine particles". In order to avoid acute lung injury, this size fraction should account for less than 0.6% of the sprayed aerosol particles. In contrast, the particle size distribution of aerosols released by hand-pump sprays containing fluorine-based or silicone-based compounds have not been investigated in Japan. Thus, the present study investigated the aerosol particle size distribution of 16 household hand-pump sprays. In 4 samples, the ratio of fine particles in aerosols exceeded 0.6%. This study confirmed that several hand-pump sprays available in the Japanese market can spray fine particles. Since the hand-pump sprays use water as a solvent and their ingredients may be more hydrophilic than those of aerosol sprays, the concepts related to the safety of aerosol-sprays do not apply to the hand-pump sprays. Therefore, it may be required for the hand-pump spray to develop a suitable method for evaluating the toxicity and to establish the safety guideline.

Keywords: hand-pump spray, fluorine-based compound, silicone-based compound, particle size distribution, inhalation

1. Introduction

Japan has published guidelines on waterproof aerosol spray to improve product safety by avoiding acute lung injury resulting from inhalation of aerosols containing fluorine-based or silicone-based compounds[1]. Furthermore, the Aerosol Industry Association of Japan introduced a voluntary regulation on waterproof aerosol sprays[2]. The guidelines and voluntary regulations define aerosol particles of diameter ≦10 μm as "fine particles"; this size fraction is limited to less than 0.6% in aerosols. It is thought that the fine particles can reach alveolar region, and then causing lung injury[1].

However, to the best of our knowledge, there have been no reports in Japan on acute lung injury due to the inhalation of aerosols sprayed from the hand-pump sprays containing fluorine-based or silicone-based compounds, and the only known case was published in Canada[3]. It was thought that the size of aerosol particles emitted from the hand-pump sprays was larger than that of aerosol sprays, and that the risk of inhalation of toxic particles was, therefore, low[4]. However, a case of acute lung injury caused by a cleaning agent was reported, where the ratio of fine particles was approximately 1%[5]. Thus, it is necessary to ascertain the particle size distribution of the aerosol particles sprayed from hand-pump sprays containing fluorine-based or silicone-based compounds in order to evaluate the safety of these sprays. However, these data have not been investigated. Therefore, in the present study, the particle size distribution of aerosol particles sprayed from household hand-pump sprays containing fluorine-based or silicone-based compounds in Japan have been determined.

To whom correspondence should be addressed:
Tsuyoshi Kawakami; Division of Environmental Chemistry, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan Tel: +81-3-3700-1141 ext.367; Fax: +81-3-3700-6950;
E-mail: tkawa@nihs.go.jp

2. Materials and methods

2. 1 Samples

Hand-pump sprays containing fluorine-based or

silicone-based compounds were purchased from several retail and online stores in Japan from April to June 2014 (Table 1). Although the constituents were not described for products A1, A4, and A7, these products were selected because the waterproofing effect was expressed on the product label. A total of 16 samples were surveyed and categorized as follows: sprays for waterproofing textiles, and kitchen and bathroom (8 samples), ironing sprays (2 samples), clothing care sprays (2 samples), and sprays to prevent adhesion of pollen to masks and clothing (4 samples).

2.2 Measurement of particle size distribution

The particle size distributions of aerosols sprayed from the hand-pump sprays were measured by a HELOS/KR laser diffraction sensor (Sympatec GmbH, Germany). The distance from the laser beam to the nozzle of the sample was fixed at 15 cm and room temperature was 25° C.

Firstly, the sampling time was examined to confirm the reproducibility of the particle size distribution by acquiring data every 10 ms. Based on the results of this preliminary experiment, the sampling time was fixed from 0 to 0.5 s.

Measurements were repeated in triplicate for each sample. The ratios of the particles with diameter ≦9 μm and 11 μm were calculated after determining the particle size distribution. (The ratio of the particles with diameter ≦10 μm was not calculated by analytical software.)

3. Results and Discussion

3.1 Particle size distributions

Representative examples of the particle distributions are shown in Fig. 1. The frequency distributions of all the samples showed a single peak and good shape, except for B1 and B2, which showed a slightly bimodal distribution. The ratios of the particles with diameter

Table 1. List of hand-pump sprays surveyed in this study.

	Sample No.	Usage	Country	Ingredients	Refractive index[a]
Waterproofing sprays	A1	Fabric	UK	Unknown	1.34
	A2	Fabric	UK	Perfluoroalkyl acrylate copolymer, sunscreen agent	1.34
	A3	Leather and fabric	Japan	Silicone, ethanol	1.34
	A4	Leather	UK	Unknown	1.34
	A5	Ceramic products, bathroom	Unknown	Silicone, water	1.33
	A6	Kitchen and bathroom	Japan	Modified silicone, fluororesin, solubilizer	1.34
	A7	Kitchen and bathroom	Japan	Unknown	1.34
	A8	Kitchen and bathroom	Unknown	Modified silicone, fluororesin, solubilizer	1.34
Non-waterproofing sprays	B1	Iron	South Korea	Water-soluble polymer, silicone, fragrance	1.34
	B2	Iron	South Korea	Water-soluble polymer, silicone, fragrance	1.34
	B3	Clothing care	Unknown	Acrylic resin, fluororesin, silicone resin	1.34
	B4	Clothing care	Unknown	Silicone, deodorant, degerming agent	1.34
	B5	Preventing pollen adhesion to masks and clothing	South Korea	Polysiloxane derivative, surfactant, ethanol, flocculant, titanium dioxide, fragrance	1.33
	B6	Preventing pollen adhesion to masks and clothing	Japan	Water, alcohol, modified silicone, antistatic agent, sticker, stabilizer, antibacterial agent, degerming agent	1.36
	B7	Preventing pollen adhesion to masks and clothing	Japan	Polysiloxane derivative, nonionic surfactant, green tea extract, pure water, ethanol	1.35
	B8	Preventing pollen adhesion to masks and clothing	Japan	Polysiloxane derivative, nonionic surfactant, green tea extract, pure water, ethanol	1.35

[a] Measured by Abbe refractometer.

≦9 μm and 11 μm are listed in Table 2. In four samples (A3, B4, B6, and B7), the ratio of particles with diameter <9 μm in aerosols exceeded 0.6%. Furthermore, in six samples (A3, A8, B3, B4, B6, and B7), the ratio of particles with diameter <11 μm in aerosols exceeded 0.6%. In contrast, the aerosol particles sprayed from five samples (A4, A5, B1, B2, and B5) contained few or no particles smaller than 11 μm. In several European countries, it is thought that the risk of an acute lung injury due to the inhalation of a hand-pump spray is lower than that associated with an aerosol spray[4]. However, this study confirmed that several hand-pump sprays available in the Japanese market can spray fine particles with diameter ≦10 μm whose ratio in aerosol particles exceed 0.6%.

3.2 Safety of hand-pump sprays

Four of the tested products were labeled as containing flurororesin (A2, A6, A8, and B3) and silicone resin (B3). The use of silicone, modified silicone, and polysilicone derivatives were described in the other products. However, it was not possible to estimate whether these silicone products were used for waterproofing. In three products (A1, A3, and A7), the ingredients were not described. It is expected that the examination of the cause of acute lung injury by using these products takes time. Furthermore, the medical treatment for acute lung injury might be delayed owing to insufficient information about the product ingredients. Thus, it is advisable that sufficient information about the ingredients of their products be provided by the manufacturers.

In case of an acute lung injury caused by waterproof aerosol spray containing fluororesin, it was thought that the fluororesin reached the alveolus, affecting the surface tension of the lung and leading to alveolar collapse[6,7]. Cases of acute lung injury by aerosol sprays containing silicone resin were also reported[8]. The experiments on animals have showed that silicone resin were less toxic than fluororesin. In addition, the toxicity of the aerosol spray containing silicone oil was not observed in the animal experiments[9,10]. However, the Silicones Environmental Health and Safety Council of North America (SEHSC) recommends that, to

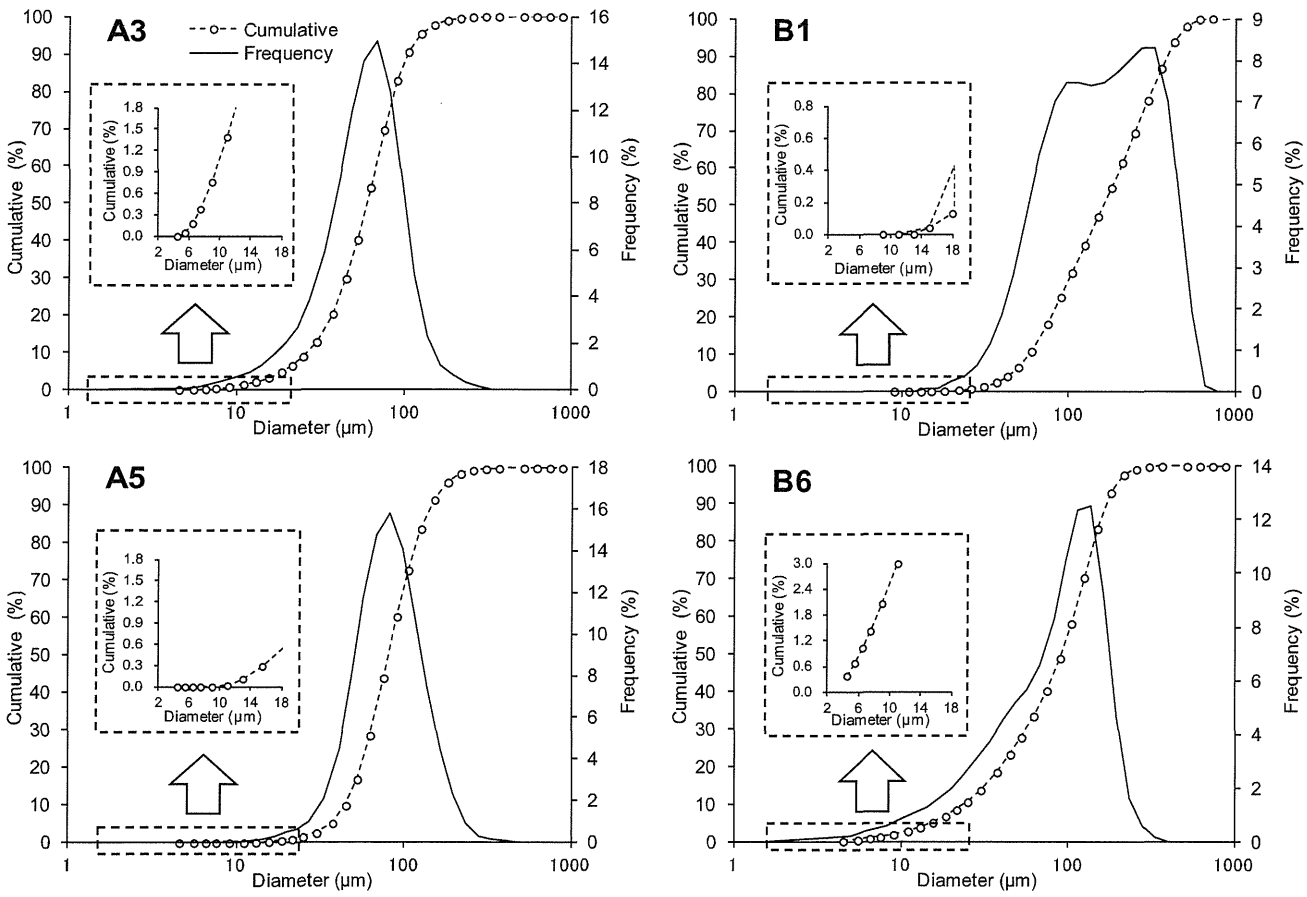

Fig. 1. Cumulative and frequency distributions of aerosol particles sprayed from samples A3, A5, B1, and B6.

Table 2. Ratio of fine particles in aerosols sprayed from hand-pump sprays.

	Sample No.	The ratio of fine particles (%)[a]	
		diameter <9 μm	diameter <11 μm
Waterproofing sprays	A1	0.1	0.4
	A2	0.2	0.5
	A3	0.8	1.4
	A4	0.0	0.1
	A5	0.0	0.0
	A6	0.0	0.2
	A7	0.3	0.6
	A8	0.4	0.8
Non-waterproofing sprays	B1	0.0	0.0
	B2	0.0	0.0
	B3	0.6	1.2
	B4	1.7	2.7
	B5	0.0	0.0
	B6	2.1	3.0
	B7	1.6	2.0
	B8	0.2	0.4

[a] Average values calculated from triplicate measurements.

prevent chemical and lipoid pneumonia, consumer aerosol application for any hydrophobic silicone-based material (such as silicone oil), regardless of the method of aerosol generation, should contain no more than 1% of particles with a diameter of 10 μm or less[11].

The aerosol particles generated from aerosol sprays become smaller over time by the evaporation of organic solvents, such that these particles can easily penetrate the alveolus[4]. In contrast, the size of aerosol particles generated from hand-pump sprays studied in this survey may not change over short time periods because these sprays use water as a solvent. Furthermore, their ingredients may be more hydrophilic than those of aerosol sprays. Thus, the concepts related to the safety of aerosol sprays do not apply directly to the hand-pump sprays. Therefore, it may be required for the hand-pump spray to develop a suitable method for evaluating the toxicity and to establish the safety guideline.

4. Acknowledgment

The authors wish to thank Mr. Hidehiko Matsuno (Japan Laser) for his technical advice during this study.

5. References

1) Office of Chemical Safety, Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare (MHLW): http://www.nihs.go.jp/mhlw/chemical/katei/manu/bousui/bousuimanual.html
2) Aerosol Industry Association of Japan (AIAJ): http://www.aiaj.or.jp/img/lm_12/aerosol_4.pdf
3) Laliberté M, Sanfacon G, Blais R: *Ann Emerg Med*, 1995;25:841-4.
4) Division of chemical products, Swiss Federal Office of Public Health (FOPH): Toxicology of waterproofing sprays, 2008.
5) Ohta K, Fujimori K, Shimatsu Y, Suzuki E, Fumitake G: *JJRS*, 2001;39:694-8.
6) Yamashita M: *The Experiment & therapy*, 1997;647:110-3.
7) Tashiro K, Matsumoto Y, Nishizuka K, Shibata K, Yamamoto K, Yamashita M, Kobayashi T: *Intensive Care Med*, 1998;24:55-60.
8) National Consumer Affairs Center of Japan (NCACJ): http://www.kokusen.go.jp/pdf/n-20130404_1.pdf
9) Yamashita M: Inhalation toxicity of waterproofing spray, Report of the grant from Ministry of Health and Welfare of Japan, 1996.
10) Yamashita M: Inhalation toxicity of waterproofing spray containing silicone oil, Report of the grant from Ministry of Health and Welfare, 1997.
11) The Silicones Environmental, Health, and Safety

Council of North America (SEHSC): http://sehsc.americanchemistry.com/Research-Science-Health-and-Safety/Guidance-for-Aerosol-Applications-of-Silicone-Based-Materials.pdf