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Safety Evaluation of Perfluoropolyethers, Liquid Polymers used in Barrier Creams and other Skin-care Products

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Abstract—Fomblin HC products are a 'family' of high-purity perfluoropolyethers manufactured for barrier cream and other personal care applications which involve direct application to the skin. To confirm the safety of such use, representative Fomblin HC products were tested in experimental animals for acute toxicity, primary and repeated insult irritancy, sensitization and photosensitization, subacute oral toxicity and comedogenicity; mutagenicity was examined *in vitro*, and irritancy or sensitization was also investigated on human skin (in patch tests with volunteers). A high molecular weight Fomblin HC only was tested in rats for subacute oral toxicity and in man for dermal effects. Single oral doses of 15 g/kg body weight were without evident toxicity to rats, as were single dermal applications or an ip injection at 5 g/kg. No primary irritant action was seen in rabbits or man, and similarly there was no evidence of skin sensitization or photosensitization in guinea pigs, or sensitization in man. No mutagenic action on *Salmonella* strains of tester bacteria was seen. In repeat dose irritancy or oral toxicity tests in rabbits or rats, no adverse effects of Fomblin HC products were noted; in particular, daily oral administration (1000 mg/kg/day) to rats over 28 days produced no significant reaction. No comedogenic action was found. From the known chemistry of the perfluoropolyethers, the test programme reported here and the limited published data, it is concluded that the intended use of Fomblin HC products in formulations applied to human skin has a high margin of safety. Copyright © 1996 Elsevier Science Ltd.

INTRODUCTION

Background: chemical and biological non-reactivity of fluorocarbons

Fluorine, the most electronegative and most strongly oxidizing of the elements, reacts particularly strongly with organic matter and substances: because of its small atomic size, it can replace hydrogen within an organic molecule, and several fluorine atoms can be fitted around a single co-ordination centre within such a molecule. Many fluorinated molecules are now used in therapeutic or herbicidal products. Perfluorination (substitution of all hydrogen atoms of an organic molecule by fluorine) of suitable organic molecules confers even greater chemical non-reactivity, and combines this with biological inertness and high solubility for gases. This was exploited by the developers of synthetic plasma substitutes ('fluorocarbon blood substitutes'): emulsions of perfluorinated alkanes or alkylamines such as FC-43 (3M Corporation tradename: Fig. 1a) and Fluosol-DA (Green Cross Corporation tradename: Fig. 1b) have been shown to possess transport capabilities for respiratory gases which make them suitable as plasma extenders for mammalian blood. These and other closely related, perfluorinated products have been evaluated for safety and plasma-extender efficacy in bacteria (mutagenicity assays), mice, rats, rabbits, dogs, pigs, macaque monkeys, baboons and man, and a number of safety reviews have been published (Biro, 1993; Bowman, 1983; Riess, 1992 and 1994; Sloviter, 1975): possible long-term effects on immune system parameters (reticuloendothelial effects affecting immunoglobulin production, complement activation giving pulmonary reactions) have been described by some workers, but some or all of these may be related to emulsifiers or other components of the tested products. Where the perfluorocarbon component itself has been implicated in adverse reaction, this has followed prolonged and high-dose exposure after direct introduction to the circulating blood. In general, the perfluorocarbon components of blood substitutes are believed to be largely or wholly inert with respect to toxicity.

Perfluorodecalin (Fig. 1c) and a range of other perfluorinated organic substances, evaluated for use as vitreous substitutes in ophthalmic surgery, are also agreed to be generally safe (Berglin *et al.*, 1993; Blinder *et al.*, 1991; Chang *et al.*, 1991; de Queiroz

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Abbreviations: FCA = Freund's complete adjuvant; PTFE = polytetrafluoroethylene; TCSA = 3,3',4',5-tetrachlorosalicylanilide.

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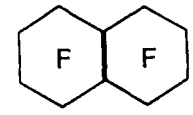
(a) $N(CF_2 - CF_2 - CF_2 - CF_3)_3$

(b) $N(CF_2 - CF_2 - CF_3)_3$

(c)



(a) Perfluoro-tri-n-butylamaine (major component of FC-43);
(b) perfluoro-tri-n-propylamine (major component of Fluosol DA with (c) below;
(c) perfluorodecalin.

Fig. 1. Molecular structures of perfluorinated organic chemicals.

et al., 1992; Eckardt and Nicolai, 1993; Green *et al.*, 1992 and 1993; Ong *et al.*, 1993; Velikay *et al.*, 1993; see also Discussion and Conclusions in this paper).

Solid perfluoropolymers are similarly stable and non-reactive. Fluoroplastics, such as tetrafluoroethylene/hexafluoropropylene copolymer and vinylidine difluoride/hexafluoropropylene, have been widely used as potable water containers, and in food- and drug-processing applications (Sheftel, 1990). Polytetrafluoroethylene (PTFE), a highly stable homopolymer, is well known for its exceptional thermal and chemical stability: the only known health hazard arising from its use is polymer fume fever, a normally transient, influenza-like reaction occurring after inhalation of fumes produced at very high temperatures (above 350°C: probably due to the thermal degradation products hydrogen fluoride and/or carbonyl fluoride, Ausimont SpA, 1995).

Perfluoropolyethers: chemistry and uses

In addition to the generally non-reactive nature of perfluorinated organics, the presence of oxygen in the 'backbone' of the perfluoropolyether molecule in the form of ether linkages confers significant advantages: since the number of possible conformational structures for the molecule is increased, perfluoropolyethers exist as liquids even under conditions of low temperature and when produced as high molecular weight polymers. The combined presence of C—F and C—O bonds confers high chemical and thermal stability. The concentration of highly electronegative fluorine atoms clustered around the central polymer chain provides a steric shield against chemical attack (including photo-oxidation). The high degree of fluorination, with consequent clustering of electronegative centres, results in low intermolecular forces.

Table 1. Properties of principal Fomblin HC products[a]

Property	HC/01	HC/04	HC/25	C/R
Molecular weight	650	1500	3200	6250
Kinematic viscosity (oSt)	1.2	40	250	1300
Fluorine content (%)	69.3	68.4	68.1	67.9
Pour point (°C)	−105	−62	−35	−25
Boiling point (°C)	140	—	—	—
Vapour pressure (mm Hg)	10	10^{-3}	10^{-5}	10^{-7}
Interfacial tension *v.* water (dynes/cm)	55	55	55	55
Surface tension (dynes/cm)	17	21	22	24
Neutralization number (mg KOH/g)	0.01	0.01	0.01	0.01
Refractive index	1.268	1.293	1.299	1.302
Density	1.76	1.87	1.90	1.91

[a]All properties measured at 20°C.

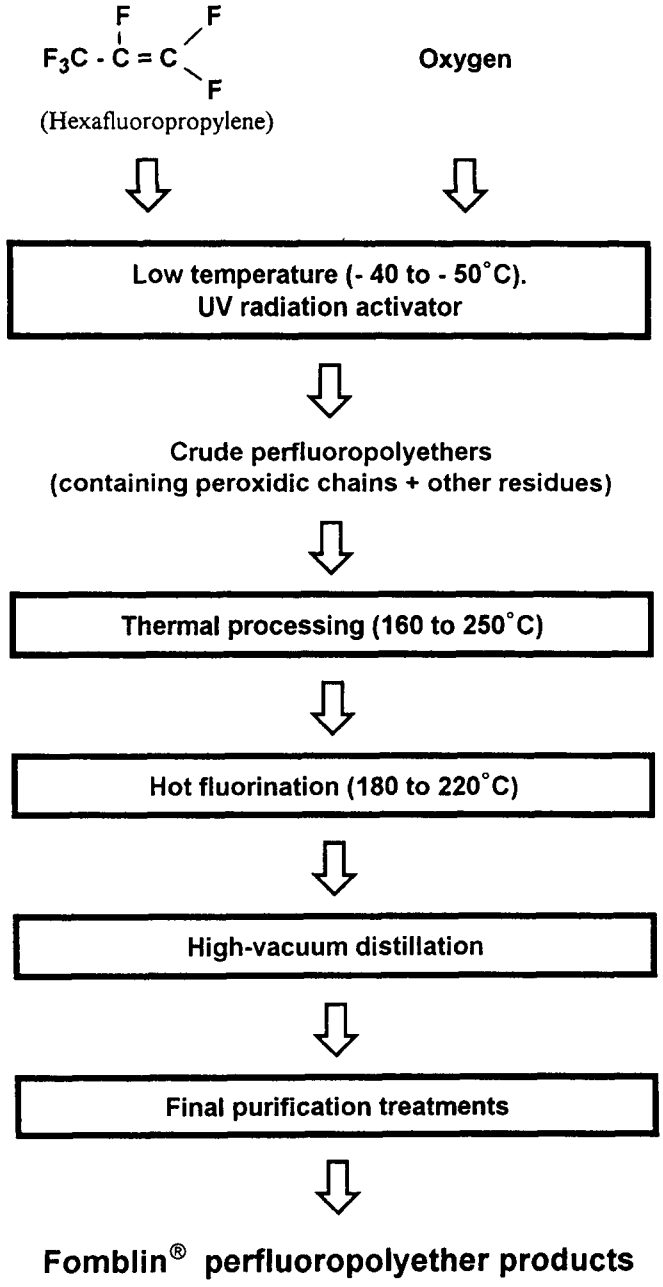


Fig. 2. Montefluos manufacturing process for Fomblin® products.

These electrochemical factors combine to produce the unusual properties by which perfluoropolyethers are characterized (see Table 1): these include high thermal stability (up to 300°C), photostability (for near-UV wavelengths, 200–380 nm, transmittance is close to 100%), hydrophobic and lipophobic properties, and high gas solubilization.

The Montefluos synthetic process shown in Fig. 2 includes chemical neutralization after fluorination, together with vacuum distillation and other proprietary final purification procedures. The final products are Fomblin perfluoropolyethers of specified viscosities, performance characteristics and average molecular weights (see Table 1: for the higher molecular weight polymers HC/04, HC/25 and HC/R, the content of low molecular weight molecules, molecular weight < 1000, is less than 0.1%). These are colourless, odourless liquids of high purity with no measurable free fluorine or inorganic fluoride. Products are characterized by spectral determinations (Fourier transform-infrared and nuclear magnetic resonance) and the absence of inorganic catalyst residues is verified. Batch purities are monitored by acidity number (KOH neutralization) and interfacial tensions with water. The chemical structure of all Fomblin products is shown in Fig. 3.

Fomblin perfluoropolyethers have wide applications as human personal-care products (designated the Fomblin HC family) and industrial lubricants (Fomblin Y products). Both fields of application rely on the formation of thin but stable films of high molecular weight perfluoropolyether material. Fomblin HC products have a primary application in the prevention and treatment of irritant dermatitis, but are also widely used in rinse-off and longer term skin-contact cosmetic products.

Control of irritant dermatitis

Chronic dermatitis of the hands is a major occupational disease: in the USA, it is estimated that dermatitis (irritant or allergic) accounts for about 30% of all illness in the workplace (Agency for Toxic Substances and Disease Registry, 1993). A Swedish investigation of 901 car mechanics found that 15% reported hand eczema within the last 12 months, and 55% of these cases were diagnosed as irritant contact dermatitis (Meding *et al.*, 1994).

While physical skin protection by the use of impermeable gloves is appropriate for certain industrial process operators, the limits placed on manual dexterity by gloves, and the discomfort caused during prolonged use, reduce the level of compliance with such procedures or render them impractical in the (common) situations where an immediate, corrosive hazard does not arise from skin contact with process chemicals. Entrapment of material (especially particulate solids) between a loose-fitting glove and the skin can also provide a hazard in itself. Many skilled and unskilled workers therefore depend on barrier creams to minimize skin contact with industrial chemicals. Before the development of Fomblin perfluoropolyethers, barrier creams commonly fell into two product classes. Water-soluble creams protect against organic solvents, paints, mineral and vegetable oils, metallic powders and many non-hydrated chemical products. Such creams have to be re-applied after each occasion of contact with water or aqueous-based products. Water-insoluble (water repellent) creams protect against mineral acids, surfactants and a range of water-based products. Re-application is normally less of a problem than for water-soluble creams, but such creams generally form an occlusive covering to the skin. This prevents local thermoregulation by perspiration, and may have a comedogenic effect through blockage of sebaceous and sweat gland orifices.

The previously described properties of Fomblin perfluoropolyethers suggested a great potential for their use in barrier formulations. Because of the high expectation of biological safety on structural grounds, preliminary efficacy evaluations of creams containing 4% or less by weight of the Fomblin product HC/R (see Table 1) were undertaken, alongside the programme of safety evaluation described in this paper. Among 22 female volunteers with chronic irritant dermatitis, marked resolution of symptoms was recorded after 40 days of three daily applications, based on subjective scoring of patient discomfort and objective evaluation of the treated skin surfaces by scanning electron microscopy (Bencini *et al.*, 1990). 25 engineering workers having severe, chronic irritant dermatitis (confirmed as non-allergic in nature) from occupational skin exposure also showed good resolution of symptoms over a 3-month period of regular application (Pantini *et al.*, 1990). In a third study (Crosti *et al.*, 1992), 40 patients suffering from chronic, non-allergic dermatitis of the hands were divided into two groups (matched for age, occupation and disease status as far as possible). Over 30 days, regular use of base cream containing 4% Fomblin HC/R caused complete resolution of symptoms in 16 of 20 patients, while use of base cream alone cured three of 20 patients. Other human trials have demonstrated protective effects of creams containing Fomblin HC/R against facial skin irritation caused by hydrogen sulfide-containing vapour (Pasquariello *et al.*, 1992) and coloration of the hands when immersed in water–eosin and ether–bromothymol blue solutions (Morganti and Randazzo, 1989).

To support widespread use of Fomblin HC materials in barrier creams and other cosmetic products, including the use of lower molecular weight Fomblin perfluoropolyethers, a programme of safety testing was developed: this was designed to permit adequate safety evaluation while minimizing the use of animals. The tests were performed in the laboratories of the Istituto di Ricerce Biomediche "Antoine Marxer" (RBM S.p.A) Italy, Huntingdon Research Centre Limited (HRC) England or Life Science Research Limited (LSR) England. UK Home Office regulations (HRC, LSR) or the CIOMS Ethical Code and EC Directive 86/609 (RBM) were followed to ensure high standards of animal welfare.

Volunteers agreeing to take part in the human patch test for irritancy and sensitization (performance of which was approved by reviewers of the test laboratory, Inveresk Research International) were fully informed of test objectives and signed an appropriate consent form accepting the test conditions prior to inclusion in the study. Arrangements for the patch test performed on human volunteers by the Japan Hair Research Institute complied with all relevant national laws and accepted consent procedures.

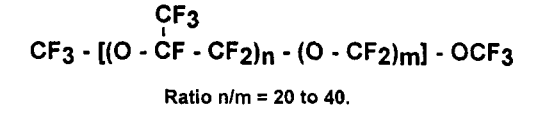


Fig. 3. Molecular structure of Fomblin® perfluoropolyethers.

Table 2. Non-human safety testing of Fomblin HC products

Fomblin product	Study type								
	Acute oral	Acute dermal	Acute ip	Skin, eye and 2-wk irritation	Oral mucosa irritation	Skin sensitization	Photo-sensitization	Comedo-genicity	Bacterial mutation
HC/25	✔	✔	✔	✔		✔	✔	✔	✔
HC/01	✔	✔		✔	✔	✔			✔
HC/04						✔			
HC/R						✔	✔		

MATERIALS AND METHODS

The Fomblin HC products used in testing are described in Table 1. The non-human phase of the safety testing programme is summarized in Table 2.

Test chemicals

A variety of different Fomblin HC products were used, all being produced to a purity greater than 99.9%. For treatment and prevention of dermatitis, HC/25 and HC/R are used: the lower molecular weight product, HC/25, was therefore selected for testing, as its lower viscosity made dose manipulation more practical and any biological activity could be expected to decrease with increasing molecular weight (for a single human volunteer test 'Fomblin Y25' sample was used: this was an early production batch of the Fomblin HC/25 product and therefore had the same general properties but was of slightly lower purity). To provide an 'extreme case' model, and because low molecular weight 'Fomblin HC Light' products are recommended for use in a wide range of cosmetic products, the lowest molecular weight product Fomblin HC/01 was also tested in several types of study. Detection of any sensitizing or photosensitizing activity on skin was accorded particularly high priority: accordingly, Fomblin products of intermediate molecular weight, and the maximum molecular weight HC/R product, were evaluated in one or both of these tests, in addition to Fomblin HC/25 and HC/01.

Acute toxicity, primary irritancy

Acute toxicity was evaluated in CD rats, by the oral and dermal routes; in addition, the toxicity of Fomblin HC/25 only was also examined using ip injection. In all cases, test procedures followed relevant EC guidelines (European Commission, 1992), and each was performed as a 'limit test' with a single group of five males plus five females. The test chemical was dosed without dilution, at doses of 15 g/kg body weight (orally) and 5 g/kg (dermally and by ip injection). Dermal exposure was for a 24-hr period (under occlusive dressings for HC/25, semi-occlusive for HC/01). Animals were observed for 14 days after dosing.

Primary irritancy to the skin, and eye irritation, were assessed in New Zealand White rabbits. To assess skin irritation test material (0.5 ml) was applied to sites with intact and abraded skin (HC/25) or intact skin alone (HC/01) which were then covered for 24 hr (HC/25: occlusive dressings) or 4 hr (HC/01: semi-occlusive dressings). To assess irritancy to the eye, a 0.1 ml volume of HC/25 or HC/01 was carefully instilled into the conjunctival sac of the right eye of each rabbit. Tests with HC/25 used six rabbits; those with HC/01 used three rabbits. Reactions were assessed at intervals up to 72 hr or 7 days after dosing. In both types of study, animals were closely monitored for discomfort or adverse reaction and relevant EC test guidelines were followed (European Commission, 1992).

Repeated application dermal irritancy

Groups of six male plus six female New Zealand White rabbits were used. On days 0 and 7 of the test period, four dorsal sites (each approx. 6 cm^2 in area) were shaved on each animal and two of these sites were abraded by careful scratching into the stratum corneum. On day 1, and each successive day to day 14, undiluted material or 0.9% saline control (0.5 ml) was applied to each pair (abraded/intact) of sites, then covered by semi-occlusive dressings. Local reactions were recorded daily, and after termination all skin sites were subjected to histopathological examination.

Fomblin HC/01 was also examined for irritant action on the oral mucosa of CD rats: test and control groups, each comprising eight male rats, were subjected to swabbing of the interior of the mouth with undiluted HC/01 and 0.9% saline, respectively. This swabbing (with 0.25 ml/occasion, duration 30 sec) was performed four times daily for 7 consecutive days. The oral mucosa and labial junctions of each animal were scored daily up to termination on the day of the last treatment. At termination, the tongue and the oral cavity/mucosa and lips ('*in situ*' with the skull), plus any macroscopic abnormalities, were fixed for histopathological examination.

Repeat dose toxicity

Possible cumulative toxicity was investigated in a subacute (4 wk) rat oral toxicity test. Study design was based on the limit test in the EC guideline B.7 (European Commission, 1992), but with satellite groups for investigation of absorption/excretion. Five male and five female CD rats were dosed orally by gavage with undiluted Fomblin HC/25 each day for

Table 3. Haematological findings in a 4-wk oral toxicity study of Fomblin HC/25 in the rat

Group and dose (mg/kg/day)	PCV (%)	Hb (g/dl)	RBC (10^6/mm^3)	MCHC (%)[a]	MCV (fl)[b]	Total WBC ($\times 10^3$/mm^3)	N	L	E	B	M	Plts ($\times 10^3$/mm^3)	TT (sec)
Male controls (distilled water)	42	14.8	6.3	35.1	67	14.0	2.70	11.06	0.06	0.00	0.13	825	24
Male HC/25 (1000) group	43	15.1	6.5	35.4	66	14.7	3.75	10.85	0.12	0.00	0.00	820	23
Female controls (distilled water)	44	14.8	6.3	33.4	71	10.7	1.43	9.08	0.12	0.00	0.07	859	19
Female HC/25 (1000) group	45	14.8	6.3	32.9	71	11.8	2.53	9.08	0.10	0.00	0.05	773	20

PCV = packed cell volume Hb = haemoglobin RBC = red blood cell count MCHC = mean corpuscular haemoglobin concentration MCV = mean corpuscular volume WBC = white blood cell count N = neutrophils L = lymphocytes E = eosinophils B = basophils M = monocytes Plts = platelet count TT = thrombotest

[a] Calculated as Hb × 100 ÷ PCV.
[b] Calculated as PCV × 10 ÷ RBC.).

Table 4. Blood chemistry of rats in a 4-wk oral toxicity study of Fomblin HC/25

Group and dose (mg/kg/ day)	Glu (mg/dl)	Total protein (g/dl)	Alb (g/dl)	Glob (g/dl)[a]	A/G (mEq/ litre)[b]	UN (mg/dl)	Creat (mg/dl)	AP (mU/ ml)	GPT (mU/ml)	GOT (mU/ml)	Bili (mg/dl)	Na (mEq/ litre)	K (mEq/ litre)	Ca (mEq/ litre)	P (mEq/ litre)	Cl (mEq/ litre)	Chol (mg/dl)
Male controls (distilled water)	80	6.4	3.1	3.3	0.96	14	0.4	415	24	62	0.1	144	4.2	5.3	5.3	98	60
Male HC/25 (1000) group	84	6.7	3.1	3.5	0.89	14	0.5	362	26	60	0.1	144	4.0	5.3	5.6	97	70
Female controls (distilled water)	89	6.7	3.3	3.3	1.01	16	0.5	224	19	49	0.1	143	4.0	5.4	4.7	99	98
Female HC/25 (1000) group	89	6.7	3.3	3.4	0.97	17	0.5	213	17	50	0.1	144	4.0	5.2	4.5	101*	86

Gluc = glucose Alb = albumin Glob = globulin A/G = albumin/globulin ratio UN = urea nitrogen Creat = creatinine AP = alkaline phosphatase
GPT = glutamic-pyruvic transaminase also known as 'alanine aminotransferase' GOT = glutamic–oxaloacetic transaminase also known as 'aspartate aminotransferase' Bili = total bilirubin Chol = cholesterol

[a] Calculated by subtraction: total protein minus albumin.
[b] Calculated from total protein and albumin concentrations.
*$P < 0.05$ *v.* controls using (Student's *t*-test).

Table 5. Microscopic pathology findings in a 4 wk oral toxicity study of Fomblin HC/25 in the rat

Tissues examined		Controls[a]	Test rats[b]	Controls[a]	Test rats[b]
		Males		Females	
Heart	Total examined	5	5	5	5
	Not remarkable	5	5	5	5
Liver	Total examined	5	5	5	5
	Not remarkable	2	1	3	4
	Minimal focal parenchymal inflammatory cells	3	4	1	1
	Focus of capsular fibrosis	1	0	1	0
	Median cleft: no abnormality detected in section examined	0	1	0	0
Spleen	Total examined	5	5	5	5
	Not remarkable	5	5	5	5
Kidneys	Total examined	5	5	5	5
	Not remarkable	5	1	5	5
	Occasional groups of basophilic cortical tubules	0	2	0	0
	Minimal focal interstitial mononuclear cells	0	1	0	0
	Dilated tubule in medulla	0	1	0	0
	Linear area of dilated/basophilic tubules	0	1	0	0
Adrenals	Total examined	5	5	5	5
	Not remarkable	5	5	5	5
Lymph nodes	No. examined	3	5	2	4
	Not remarkable	0	0	0	0
	Cervical: minimal reactive hyperplasia	3	5	2	4
	Deep cervical: minimal reactive hyperplasia	0	1	0	0
Skin	Total examined	0	1	0	0
	Not remarkable	0	0	0	0
	Area of healing ulceration with scab	0	1	0	0
Stomach	Total examined	0	0	3	1
	Not remarkable	0	0	2	0
	Focus of ectopic nonglandular mucosa	0	0	1	1

[a] Dosed only with water.
[b] Dosed with HC/25 1000 mg/kg/day.

28 days (administered dose 1000 mg/kg/day); a similar control group of rats was dosed daily with distilled water only, and two satellite groups (each three males, three females: one group dosed with HC/25, one with distilled water) were also established. Animal behaviour and appearance were observed daily until termination on the day after the last doses were given (satellite animals were dosed and kept alive for up to 30 days). Blood samples for haematological and biochemical analysis were taken (from the orbital sinus) from fasted main group animals on day 25 of the dosing period and urine samples were collected overnight on day 28/29 (with food and water removed). Animals of the satellite group were cannulated for bile collection at the end of the test period: samples were taken from one male plus one female of each group over the time periods 0–4, 4–8 and 8–24 hr after dosing on day 28 and this was repeated with two more pairs of animals on day 30. All animals were weighed weekly, and food consumption was monitored on a per-cage (single sex, three or five rats) basis. At termination, blood was withdrawn (under anaesthesia) by cardiac puncture from all main group animals and transferred to heparinized tubes; subsequently these animals were killed by carbon dioxide inhalation, and complete autopsies followed. Adrenals, kidneys, livers, ovaries or testes were weighed, and the first three of these plus hearts, spleens and macroscopically abnormal tissues were subjected to histopathological examination using light microscopy.

Urine and terminal blood samples from main group test and control animals, plus the bile samples (24: eight rats × 3 collection times) from satellite animals, were frozen (at approx. − 20°C) immediately after collection. Some months later, after development and validation of a suitable analytical method, levels of Fomblin HC or its metabolites in these samples were determined by measurement of fluoride levels. The analyses were performed by a two-stage procedure. Each sample was first burnt under controlled conditions, in an atmosphere of steam, and the resultant vapour stream was collected by condensation the level of fluoride ions in this condensate was determined by ion chromatography. The initial pyrohydrolysis step used procedures previously developed in the laboratories of Montedison S.p.A (Istituto Guido Donegani) for release of fluoride from complex matrices such as PTFE polymer: the combustion process is precisely controlled by the use of a platinum sample container in a ceramic oven at 1100°C (oxygen flow 0.2 litres/min). This releases organic or inorganically bound fluorine atoms as fluoride ions, and subsequent ion chromatography using AG 4A + A5 4A columns, with aqueous 70 mM sodium borate solution as eluent and a conductivity detector, gives a limit of fluoride detection of 2 ppb (measured as F^- in aqueous sodium fluoride solution).

Mutagenicity testing

The bacterial mutation assay of Ames (1975) was employed, using *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100. Fomblin samples HC/25 and HC/01 were both tested at concentrations in the range 50 to 5000 μg/plate. The high molecular weight product HC/25 was tested as a mixture in dimethyl sulfoxide; HC/01 was

dissolved in CFC 133 (trichlorotrifluoroethane) for use in testing. In each case, the relevant EC test guideline was followed (European Commission, 1992).

Skin sensitization

The sensitizing potential of a range of different Fomblin HC products was evaluated: HC/R, HC/25 and HC/04 were investigated in Dunkin–Hartley albino guinea pigs using the maximization test method of Magnusson and Kligman (1969). Fomblin HC/01 was evaluated in animals of the same species and strain, but using the non-adjuvant method of Buehler (Ritz and Buehler, 1980). In all cases, the relevant EC test guideline (European Commission, 1992) was followed; the non-irritant properties of the Fomblin products allowed their use in undiluted form. For maximization tests, first inductions (intradermal injections) comprised: anterior site, Freund's complete adjuvant (FCA: 0.1 ml); middle site, Fomblin HC (or distilled water for controls: 0.1 ml); posterior site, Fomblin HC/FCA (or distilled water/FCA: 2 × 0.05 ml or 0.1 ml). 7 days later (and 1 day after skin treatment with 10% lauryl sulfate in petrolatum), 0.6 ml volumes of Fomblin were applied once, topically (under occlusive patches) for 48 hr; control animals received 0.6 ml distilled water. At challenge, 14 days later, 30 μl Fomblin on a 10-mm diameter Al-test patch (Imeco AB) was applied under occlusive dressings for 24 hr. Test and control groups each comprised 10 male plus 10 female animals. In the non-adjuvant test, 0.5 ml volumes of Fomblin HC/01 were applied topically under occlusive patches for 6 hr for each of the three inductions, and challenge exposures (15 days after the last induction) were similar but lasted 24 hr. Test and control groups each comprised 10 male animals, and controls were treated only at challenge. All sensitization tests were performed in laboratories constantly undertaking such studies, where positive control tests (using known sensitizers) were completed at intervals to confirm assay sensitivity.

The photosensitizing potentials of Fomblin HC/R and HC/25 were evaluated in Dunkin–Hartley albino guinea pigs using maximization test procedures based on the methods of Morikawa *et al.* (1974) and Maurer (1983). After an initial (day 1) intradermal injection of FCA, animals of each study were given 11 separate induction treatments (every second day over days 1–21), each comprising topical applications of 0.1 ml of the selected Fomblin product onto depilated and stripped skin followed (after 20 min) by UV irradiation. Irradiation comprised mixed UVA/UVB for 15 min (radiant exposure 3.2–3.8 J/cm^2) then UVA alone for 120 min (radiant exposure 25.2–28.1 J/cm^2). Control animals were untreated during the induction phase of the studies. Challenge on day 35 comprised topical application of 0.05-ml volumes of Fomblin solution to duplicate sites of each animal; other (positive and negative control) sites were also marked. The known photoactive agent 3,3',4',5-tetrachlorosalicylanilide (TSCA) was used as a positive control agent. 20 min later, one of each pair of test or control sites was irradiated (as for induction) while the replicate site was covered to prevent irradiation. Reactions to challenge were assessed 24, 48 and 72 hr after challenge began. Test and control groups each comprised five males plus five females.

Fomblin products were tested in human volunteers to investigate the effects of prolonged skin contact. A closed patch irritation study was performed in Japan: 32 human volunteers (20 male, 12 female, ages 20–69 yr) were exposed to undiluted Fomblin HC/25 in Finn chambers (Epitest) placed on the upper arm for 48 hr. Skin reactions were observed shortly (30 min) after patch removal, 24 hr later and 7 days after removal. In a separate (European) study, Fomblin Y25 was examined for both irritancy and sensitizing action. In this case, following a preliminary irritancy check in four volunteers, 30 volunteers took part in a study following the method of Kligman (Kligman and Wooding, 1967). Five separate, 48-hr induction treatments were given over a 3-wk period: on each occasion, 0.5 ml Fomblin Y25 was placed on a patch secured to the upper left arm of each volunteer and covered by occlusive tape. 2 wk later, challenge exposures were applied by similar patches placed on both left and right upper arms of each volunteer. Local reactions were scored 1–2 days after removal of induction patches and 1 hr plus 48 hr after challenge patch removal. Of the 30 human volunteers, 26 were female (21–65 yr) and four male (31–65 yr): three failed to complete both induction and challenge phases and were excluded.

Comedogenicity

Fomblin HC/25 was evaluated for comedogenic action (induction of 'blackheads') by repeated application to the epidermis of the rabbit ear, following the method of Kligman and Kwong (1979). Six male New Zealand White rabbits were used: 1 ml undiluted test material was applied daily for 14 days to the undersurface of the right pinna adjacent to the ear canal. Local reactions were assessed visually each day, and at termination ear sections were taken: strips of epidermis from the test and control (left ear) sites were prepared and examined under a stereomicroscope.

RESULTS

The expected low acute toxicity of Fomblin HC products was confirmed: by the oral route neither HC/25 nor HC/01 produced significant signs of reaction (clinical signs, body weight change or macroscopic abnormality at autopsy), even at 15 g/kg body weight. Dermal application at 5 g/kg was similarly non-toxic, as was ip injection of HC/25 at 5 g/kg. Perineal staining was seen in seven of 10 rats 2–3 hr after oral dosing with HC/25: this was most

probably due to anal discharge of the Fomblin which had passed directly through the gastrointestinal tract.

Both of the tested Fomblins were wholly non-irritant in the rabbit studies: a single test rabbit of the HC/25 skin irritation study showed transient, very slight erythema at the abraded skin application site, but no other signs of reaction were seen in this or the HC/01 skin test. In the eye irritation studies, no reactions to test material instillation were noted and examinations at 1–72 hr after treatment found no conjunctival, iridial or corneal changes.

Repeat dose dermal irritation tests confirmed the non-irritancy of Fomblin HC/25 and HC/01: visual evaluation of test sites found no reactions to HC/01 treatment, and only a single case (one female rabbit, abraded test site, slight erythema on days 9–14) of reaction at a site exposed to HC/25. Similar slight erythema was seen at termination at three control sites (abraded skin). No behavioural or other reactions were noted in the study animals. Animals were not restrained during dermal exposure, to minimize stress: as a consequence, some rabbits in the study conducted first (HC/25 test) interfered with their porous tape/gauze patch dressings and ingested dressing material. This led to observation of gauze masses in the stomachs of eight animals at autopsy: seven of these animals, plus one other, showed consequent, minor, mechanical damage within the stomach (areas of slight mucosal haemorrhage or ulceration). Application of tape and patches in the second (HC/01) study was adequate to prevent this problem. Microscopic examination of all test and control sites revealed a general pattern of slight inflammation of the dermis with slight epidermal inflammation or hyperplasia. These changes were minor in nature, and no pattern of increased incidence of severity in test (intact or abraded skin) sites over control sites was found: the changes were attributed to the mechanical effects of shaving, reshaving, intentional skin abrasion (where relevant) and repetitive bandaging.

In the rat, repeated application of HC/01 to the oral mucosa had no detectable effect on the behaviour or well-being of the animals; visual examination of the application sites found no erythema or other changes, and microscopic examination of sections prepared from the oral mucosa, lips and tongue identified no abnormalities. The only abnormalities observed in this study were in the spleens of two test group rats: these organs were seen at autopsy to have whitish areas, and were found to show slight focal changes (mainly capsular) of a type previously noted by the same test laboratory in untreated rats of the same age and strain. The changes were considered incidental.

In the 4-wk oral toxicity test, rats showed no visible changes in behaviour or well-being after daily dosing with HC/25 at 1000 mg/kg: body weight gains over the test period were unaffected, and there was little or no effect on food consumption. Haematological investigations of blood samples included a range of morphological and functional assessments (see

Table 6. Mean fluoride levels in the body fluids of rats in a 4 wk oral toxicity study of Fomblin HC/25

Group/ treatment	Urine (five animals/group)		Bile collection (hr) (two animals/group)						Blood (five animals/group)	
			0–4	4–8	8–24	0–4	4–8	8–24		
	Males	Females	Males			Females			Males	Females
Controls	5.59 ± 1.05	5.66 ± 2.84	17.15 ± 0.21	34.17 ± 5.48	31.70 ± 5.65	20.80 ± 6.64	36.02 ± 10.42	26.57 ± 8.16	0.77 ± 0.52	0.63 ± 0.62
Fomblin HC/15 (1000 mg/kg/day)	6.06 ± 1.24	4.37 ± 1.12	13.22 ± 0.31	30.45 ± 1.20	29.15 ± 0.63	18.52 ± 3.85	36.97 ± 9.01	30.30 ± 3.67	0.54 ± 0.32	0.51 ± 0.17

Values shown as means ± SD (μg/ml).

Table 7. Fomblin HC products, photosensitisation studies: challenge reactions in the guinea pig

			Irradiated sites				Non-irradiated sites			
			Animals showing erythema[a]				Animals showing erythema[a]			
Group	Treatment	No. of animals	24 hr	48 hr	72 hr	Total no. of responders	24 hr	48 hr	72 hr	Total no. of responders
Negative control	Fomblin HC/R	10	0	0	0	0	0	0	0	0
	TCSA	10	0	0	0	0	0	0	0	0
Test	Fomblin HC/R	10	1	1	1	1	0	0	0	0
Positive	TCSA	10	8	6	3	8	2	0	0	2
control	Ethanol	10	0	0	0	0	0	0	0	0
Negative control	Fomblin HC/25	10	0	0	0	0	0	0	0	0
	TCSA	10	0	1	0	1	0	0	0	0
Test	Fomblin HC/25	10	1	0	0	1	0	0	0	0
Positive control	TCSA	10	6	6	2	8	1	1	1	1
	Ethanol	10	0	0	0	0	0	0	0	0

[a] Slight erythema or a more marked response.

Table 3). Biochemical measurements (see Table 4) included recognized markers for hepatocellular (aspartate and alanine aminotransferase), hepatobiliary (alkaline phosphatase), or kidney (urea nitrogen, creatinine) damage. Statistical tests were applied to compare all measured parameters of the test and control groups. As the group mean values of Table 3 and Table 4 show, none of these investigations found a significant effect of HC/25 administration: a minor variation in blood chloride level caused statistical significance when a simplistic (Student's *t*) method of analysis was applied, but examination of individual animal data and the small size of the apparent increase in HC/25-dosed females showed that this was clearly a chance finding. At autopsy, occasional observations of macroscopic abnormality were recorded (enlargement of cervical lymph nodes or appearance of a nodule in the stomach mucosa in some animals). However, these abnormalities were of a nature and frequency which indicated no toxicological significance, and no pattern of difference between test and control animals was evident. Evaluation of organ weights (expressed relative to body weight) showed no effects of HC/25 treatment. Microscopic examination of the selected organs and macroscopic abnormalities also revealed no changes considered to be treatment related (see Table 5). The only apparent difference in test over control animals related to microscopic findings in the kidney tubules of male rats: four of five test group males showed some basophilic and/or dilated tubules compared with none in five controls. Such changes are generally not common in CD rats of this age, and basophilic tubules can be associated with mild degenerative change in the kidney following high-dose administration of a test chemical. However, it was concluded that the observations in this study were of no toxicological importance because only three of five males and no females showed (localized) basophilia, there were no effects on kidney weight and blood chemistry indicated no kidney damage (from creatinine, urea levels).

The conclusion of non-toxicity in this study was supported by determinations of fluoride levels in urine, blood and bile (Table 6). In all cases, measured levels were both low and closely similar in control and HC/25-treated animals. The reasons for the differences in fluoride concentrations between bile and blood seen in both test and control rats are not known; however, the results indicate an effective absence of Fomblin HC/25 (or degradation products) from circulating blood and excretory fluids. Although a more complex regimen of timed sample-taking after one or a few doses of test material would commonly be used to fully investigate absorption and bioelimination, the 'negative' results obtained in this study are strongly indicative of non-absorption of HC/25.

Fomblins HC/01 and HC/25 gave no indication of mutagenicity in bacterial mutation tests. Solvent and positive (known mutagen) plates confirmed the expected patterns of test strain growth, the inactivity of the solvents used and the sensitivity of the test procedures to mutagens.

All four Fomblin products were wholly inactive in skin sensitization tests: at challenge, no reactions were seen in any test (or control) animals. Assays for photosensitizing action also confirmed the inactivity of Fomblins HC/25 and HC/R (see Table 7): in each case only a single animal of the test group showed slight erythema at the irradiated Fomblin challenge site, and only with Fomblin HC/R did this persist for more than 24 hr. Such a low incidence of apparent response cannot be taken as evidence of photosensitization. The positive control agent TCSA showed clear and reproducible activity (an important validator of the two studies, as photosensitization tests are known to be of variable sensitivity).

In human patch tests, Fomblin Y25 and HC/25 were again inactive on the skin: in the Japanese irritancy test, no reactions were observed either immediately (30 min) after patch removal or subsequently (up to 7 days later). In the larger European study, a single volunteer showed slight dryness at the application site of the second induction

patch and another showed slight erythema at the site of the fourth induction patch; these reactions were transient and no other responses to induction or challenge were seen.

The rabbit comedogenicity test found no reactions to repeated HC/25 applications. No adverse reactions or macroscopic signs were noted among the six test animals; the only abnormalities noted during microscopic examination of the epidermal samples was slightly increased hyperkeratosis seen in both control (untreated) and test ears of a single rabbit.

DISCUSSION AND CONCLUSIONS

The present programme of safety evaluation began with consideration of the physicochemical and toxicological background to the development of perfluoropolyethers, as described earlier in this paper. This led to a high expectation of biological non-reactivity. In addition, during the course of the safety evaluation reported here, results of an acute inhalation toxicity test on an industrial grade (low molecular weight, Fomblin Y-type) perfluoropolyether became available. Rats held in a whole-body exposure chamber with an atmosphere containing 8.3% (v/v) perfluoropolyether (1328 mg/litre) for 4 hr showed no immediate or delayed adverse reactions.

A limited amount of perfluoropolyether safety evaluation has been published by other workers. One report of low acute and subacute toxicity (Starek *et al.*, 1977) is of uncertain relevance to Fomblin HC products as the similarity of chemical identity and purity of the perfluoropolyether used to that of the Fomblin HC group of materials is unknown. Other reports of localized tissue effects in the eye refer to changes seen 1 month or longer after insertion of the test materials into the eye as vitreous replacements (Eckardt *et al.*, 1991; Miyamoto *et al.*, 1984; Moreira *et al.*, 1992): the observed changes may have been contributed to by the mechanical trauma of vitrectomy, and no applications related to ophthalmic surgery are proposed for Fomblin HC products.

The general toxicity profile of perfluoropolyethers is thus favourable: the few previously discussed adverse effects of these or related perfluorocarbon materials arose at high exposure levels and under conditions not applicable to the intended use of Fomblin HC products. The findings of the test programme reported here (performed almost exclusively on products produced to the high purity specification now applied to the Fomblin HC commercial products) confirm the low toxicity of these perfluoropolyethers, and justify the limited scale of animal testing undertaken. On the basis of the observed inactivity of both high and low molecular weight Fomblins (HC/25 and HC/01) in acute toxicity, primary and repeated application irritancy and sensitization tests, plus the bacterial mutagenicity tests, it is concluded that the Fomblin HC family of products has a high margin of safety when used in human personal-care products. For the most demanding (and probably most beneficial) product application, namely use in barrier creams for constant use by industrial process workers, the high safety margin has been further verified by demonstration of an absence of photochemical (photosensitization) hazard, verification of exaggerated human skin exposure without reaction and demonstration of inactivity in the rat even after repeated high-dose ingestion. The apparent absence of Fomblin HC absorption from the gastrointestinal tract in this rat study suggests that dermal absorption in man will be minimal. Hence, delayed or subtle effects such as low-level bioaccumulation, which might have required subchronic or chronic toxicity testing for complete evaluation, are unlikely to pose a significant hazard for the intended topical use of Fomblin HC products.

Handling of Fomblin HC products as manufactured should therefore present no specific toxicological hazard: only at extremely high temperature (> 300°C, giving a possibility of 'polymer fume fever') need specific precautions be taken.

The margin of safety for use of the higher molecular weight Fomblin HC products in barrier creams, at the intended inclusion levels of 1–5%, is extremely high: there are no foreseeable adverse reactions which could limit such use, and no reasons to consider further safety testing. Other intended product applications, including use of the various Fomblin HC materials in non-ingested cosmetic and skin-care formulations, also give rise to no significant safety concerns.

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