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Toxicological Evaluation
White Out Food Grade Formula

Quotation:
ZD75PH210008-02

Sponsor:
UR Fog srl
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1 Summary

The present toxicological evaluation was carried out in order to determine the toxicological profile of "White Out Food Grade Formula when used according to its intended use.

The composition of the product is reported in the following table:

	CAS	Percentage [%]
Dipropylene glycol	25265-71-8	[redacted]
Polyethylene glycol 200	25322-68-3	
Water	7732-18-5	
Ethanol	64-17-5	
Isopropanol	67-63-0	

Table 1 - Product formula

The product is used as a fogging system therefore, during the use, the relevant toxicological endpoint to be evaluated are: skin irritation, eye irritation, sensitization, acute systemic toxicity by inhalation and ingestion.

The product could be considered slightly irritant for the skin, moderately irritant for the eye, non-skin sensitizer and practically non-toxic after acute inhalation and ingestion.
The irritation could be considered transit when the product is used according to manufacturer's instructions.

2 Introduction

The study was performed at the Test Facility Eurofins Biolab S.r.l. of Vimodrone (MI) – via B. Buozzi n. 2 (Italy).

Experimentation	Start	End	Researcher
Toxicological evaluation	03/02/2021	07/03/2021	P. Pescio

The activity was performed at Eurofins BioPharma Product Testing. The laboratories are accredited by the several Italian and international institutional accreditors, in particular Accredia (National System for the Accreditation of Laboratories) for ISO/IEC 17025.
The complete accreditation list can be found:
https://www.eurofins.it/pharma/accreditamenti/accreditations/
The present toxicological evaluation was performed to assess the health effects of the product as per EN ISO 16000-1:2006 (ISO 16000-1:2004).

3 Evaluated product

Name	White Out Food Grade Formula		
Composition	Substance	CAS	Percentage [%]
	Dipropylene glycol	25265-71-8	[redacted]
	Polyethylene glycol 200	25322-68-3	
	Water	7732-18-5	
	Ethanol	64-17-5	
	Isopropanol	67-63-0	

Table 2 – Description of the evaluated product

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4 Toxicological evaluation

The relevant toxicological endpoint to be evaluated are: skin irritation, eye irritation, sensitization, acute systemic toxicity by inhalation and ingestion.

	CAS	% w/w	Min % w/w	Max % w/w
Dipropylene glycol	25265-71-8	[redacted]		
Polyethylene glycol 200	25322-68-3			
Water	7732-18-5			
Ethanol	64-17-5			
Isopropanol	67-63-0			

Table 3 – Product composition with theoretical range

Toxicological information for each constituents is reported below.

4.1 Dipropylene glycol CAS: 25265-71-8

4.1.1 Acute Toxicity

Dipropylene glycol is not acutely toxic by oral, dermal, or inhalation exposure. Acute oral toxicity has been examined in the rat, mouse, and guinea pig and the reported LD50s were 15.8 ml/kg (16000 mg/kg), >2000 mg/kg and 17600 mg/kg, respectively. The study that provides the best documentation of design and results was reported by Spanjers and Til, 1980. The authors dosed 5 groups of animals with graded amounts of dipropylene glycol. There was no indication of clinical signs in the report, but the necropsy examination after death or at the end of the 14-day observation period was reported to have found no gross alterations of the internal organs. The authors determined an LD50 for the study of 15.8 ml/kg (approximately 16000 mg/kg). In a study designed to assess micronuclei, 6 male mice received two consecutive daily doses of dipropylene glycol via oral gavage; survivors were terminated 24 hours later. There were no deaths in the study; therefore, the LD50 for this study was >2000 mg/kg (Dow, 1999).

Dipropylene glycol vapor and aerosol has been examined for acute inhalation toxicity. An aerosol atmosphere of 6000 to 8000 mg/m^3 dipropylene glycol was not lethal to rats or guinea pigs (Oettel and Hofmann, 1961), but vaporized degradation products produced by heating dipropylene glycol to 170 °C was lethal to 5 of 6 rats exposed for 8 hours. No mortality occurred from vapors generated at 120°C. Pathologic abnormalities were not observed in any of the animals (Oettel and Hofmann, 1961).

Dipropylene glycol did not produce deaths when administered to the skin of animals. Rabbits that were reportedly administered 5000 and 20000 mg/kg dipropylene glycol to their skin did not die from the treatment (BIBRA, 1991; Opdyke, 1978; Deichman and Gerarde, 1969). Details of these studies are not available.

Based on the above acute data, dipropylene glycol is practically non-toxic by the oral, inhalation and dermal routes.

4.1.2 Irritation and Sensitization

Dipropylene glycol is slightly irritating to the skin and eyes. Dipropylene glycol was described as slightly irritating to rabbit skin in a report that did not provide details on test conditions or results (BIBRA, 1991). Similar minimal skin effects were seen in a human volunteer study where 0.2 ml of 25% dipropylene glycol in water was applied semiocclusively to 33 subjects for 24 hours. Nine subjects had mild erythema at either 30 minutes or 24 hours; two had mild erythema at both 30 minutes and 24 hours. Twenty-two subjects had no reaction after 30 minutes or 24 hours (Acklin and Plaza, 1995).
Dipropylene glycol was reported to be mildly irritating when it was placed full strength in rabbit eyes, but only transient eye irritation was reported from a formulation containing 7.2% dipropylene glycol (BIBRA, 1991). The details of the study were not provided.
Dipropylene glycol appears to have low potential to produce allergic skin reactions. There are no experimental animal studies reported, but there is a human clinical study. Adermatology clinic tested 503 (212 men, 291 women) consecutive patients with eczema for sensitivity to dipropylene glycol, using 1 to 10% dipropylene glycol applied for 2 days. This was not a standard sensitization test because there was no specific induction phase and the subjects at the onset of the study were not clinically normal. One individual was found to be sensitized; 22 had questionable erythema; and 480 were unreactive (Johansen et al., 1995).

4.2 Polyethylene glycol 200 CAS: 25322-68-3

4.2.1 Acute toxicity

4.2.1.1 Acute oral toxicity

The acute oral toxicity dose (LD50) was considered based on different experimental study report and other studies conducted on rats and mice for the test chemical. The LD50 value is >2000 mg/kg bw for acute oral toxicity. Thus, comparing this value with the criteria of CLP regulation, the given test chemical cannot be classified for acute oral toxicity.

4.2.1.2 Acute Inhalation Toxicity

The study need not be conducted because exposure of humans via inhalation is not likely taking into account the vapour pressure of the substance and/or the possibility of exposure to aerosols, particles or droplets of an inhalable size. In accordance with column 2 of Annex VIII, this end point was considered for waiver since the vapour pressure of test chemical is low and so exposure by the inhalation route is unlikely.

4.2.1.3 Acute Dermal toxicity

The acute dermal toxicity dose (LD50) was considered based on different experimental study reports conducted on rats for the test chemical. The studies concluded that LD50 value is >2000 mg/kg bw, for acute dermal toxicity. Thus, comparing this value with the criteria of CLP regulation, the given test chemical cannot be classified for acute dermal toxicity.

4.2.2 Irritation and sensitisation

4.2.2.1 Skin irritation

The dermal irritation potential of target chemical was assessed in various in-vivo experimental studies. Based on the available studies, it can be concluded that the test chemical is unable to cause skin irritation and considered as not irritating. Comparing the above annotations with the criteria of CLP regulation, it can be classified under the category “Not Classified".

4.2.2.2 Eye irritation

The ocular irritation potential of target chemical was assessed in various in-vivo experimental studies. Based on the available studies, it can be concluded that the test chemical is unable to cause eye irritation and considered as not irritating. Comparing the above annotations with the criteria of CLP regulation, it can be classified under the category “Not Classified".

4.2.2.3 Sensitisation

The skin sensitization potential of test chemical was assessed in various experimental studies conducted on human subjects and rabbits. Based on the available data for the test chemical, it can be concluded that the test chemical is unable to cause skin sensitization and thus can be considered as not sensitizing. Comparing the above annotations with the criteria of CLP regulation, it can be classified under the category “Non-Skin Sensitizer”.

4.3 Water CAS: 7732-18-5

For the purpose of this evaluation, water was not assessed because of the inherent low risk.

4.4 Ethanol CAS: 64-17-5

4.4.1 Acute toxicity

4.4.1.1 Inhalation

In acute inhalation studies, ethanol has shown a low order of acute toxicity. An LC50 value was not achieved at exposures of up to 60,000 ppm for 60 minutes in study in CD-1 mice (Moser, 1985). Mice in this study experienced moderate ataxia, which reversed after more than 4 hours recovery period at all exposure levels.

4.4.1.2 Dermal

No acute dermal toxicity was reported in a study in rabbits, LDL0=20,000 mg/kg (Monick, 1968) and although this study is not experimentally robust, the result is consistent with the finding that ethanol uptake through intact skin is poor. No other dermal study or reported result has been identified.

4.4.1.3 Oral

Ethanol has a low order of toxicity in animals following single oral exposure. Robust figures are: LD50=8300 mg/kg (oral, mouse) (Bartsch, 1976) and LD50=15010 mg/kg (oral, rat) (Youssef, 1992). An age-related difference is reported (Wiberg, 1970) in which young rats (100 days old) were less sensitive than old rats (10-12 months old) with an LD50=11,000 mg/kg versus 7,000 mg/kg. The main symptoms of acute exposure are those typical of substances which cause central nervous system depression e.g. inebriation, gait disturbance

and dose-related decrease in response to painful stimuli, respiratory depression and coma. Deaths were due to cardio-respiratory failure.

4.4.2 Irritation and sensitisation

4.4.2.1 Skin Irritation

Studies in Animals

There is little evidence of skin irritancy in animal studies. A study conducted to OECD 404 standards in rabbits showed ethanol to be not irritating (Jacobs, 1992) which agrees with an earlier study (Phillips, 1972).

Studies in Humans

In the form of biocidally active surgical spirit (70-80% ethanol in water), there is a considerable history of dermal application of ethanol as an antiseptic with no concern for skin irritancy. Similarly, large amounts of ethanol are used in a variety of cosmetics, personal care and household cleaning products.

4.4.2.2 Eye Irritation

Studies in Animals

Available data from animal studies indicates that ethanol is moderately irritating to the eye. The most recent data indicates that, when assessed in an OECD 405 study, only mild redness and chemosis remained in by day 7 with all symptoms having disappeared by day 14 (ECETOC, 1998). An older study similarly concluded that ethanol is moderately irritating (Jacobs, 1987).

Studies in Humans

In humans, direct contact of liquid ethanol on the human eye causes an immediate sensation of burning and stinging, accompanied by reflex closure of the eye. The acute discomfort subsides rapidly, although foreign body type discomfort may be felt for a day or so. Recovery is complete.

4.4.2.3 Respiratory Tract Irritation

In humans, a concentration of 5000 ppm vapour is quoted as irritating and uncomfortable to breathe but tolerable (Lester, 1951). Much higher concentrations than this would induce lachrymation and coughing.

4.4.2.4 Concusion

Ethanol is moderately irritating to the eyes but not irritating to skin. At high vapour concentrations, in air, ethanol is irritating to breathe.

4.4.3 Sensitisation

Studies in Animals

Skin Ethanol (75 % v/v) was used as solvent in the induction phase of a Magnusson and Kligman sensitization test of a polyalkalene glycol. No skin reactions were evoked at challenge with the polyalkalene glycol in 75 % ethanol in either test or control group animals (BP Chemicals, 1984). No increase in ear thickness was recorded following challenge application of ethanol in a mouse ear swelling test (Descotes, 1988).

Studies in Humans Skin
A literature review demonstrated that ethanol can be an allergen in immediate and delayed hypersensitivity by external or internal exposure and can produce subjective irritation, irritant contact dermatitis and non-immunologic contact urticaria (Ophaswongse, 1994). However, the widespread use of ethanol in cosmetics and in skin antiseptic formulations suggests that skin sensitization is not an end point of concern.

4.5 Isopropanol CAS:67-63-0

4.5.1 Acute Toxicity and Primary Irritancy

Isopropanol has a low order of acute toxicity.
It is irritating to the eyes, but not to the skin.
Very high vapor concentrations are irritating to the eyes, nose, and throat, and prolonged exposure may produce central nervous system depression and narcosis.
Human volunteers reported that exposure to 400 ppm isopropanol vapours for 3 to 5 min. caused mild irritation of the eyes, nose and throat. Although isopropanol produced little irritation when tested on the skin of human volunteers, there have been reports of isolated cases of dermal irritation and/or sensitization.
The use of isopropanol as a sponge treatment for the control of fever has resulted in cases of intoxication, probably the result of both dermal absorption and inhalation. There have been a number of cases of poisoning reported due to the intentional ingestion of isopropanol, particularly among alcoholics or suicide victims. These ingestions typically result in a comatose condition. Pulmonary difficulty, nausea, vomiting, and headache accompanied by various degrees of central nervous system depression are typical. In the absence of shock, recovery usually occurred.

The mean potential intake from all sources of propan-2-ol has been estimated to be 1.3 mg/kg bw/day in UK adult consumers, i.e. below the ADI. However, the estimated 97.5th percentile of potential intake in consumers from all sources was 5.4 mg/kg bw/day, exceeding the ADI. Exposure estimates based only on the consumption of regular carbonated drinks in teenagers from 5 EU cities lead to a potential intake of 1.4 mg/kg bw/day at the mean of the total surveyed population. Potential intakes at the 97.5th percentile varied according to the cities reaching up to 10 mg/kg bw/day at the 97.5th percentile. The ADI of 2.4 mg/kg bw would be reached for a 60 kg adult by the daily consumption of 240 ml of soft drink which is less than a normal sized can or bottle.

4.5.2 Sensitisation

The skin sensitisation potential of 2-propanol was assessed in a study performed according to OECD Guidelines for the Testing of Chemicals No. 406 and in compliance with GLP in male and female Hartley guinea pigs (Hill Top Research, Inc, 1980). In the study, 10 animals/sex comprised the 2-propanol test group and 5 animals/sex comprised the control group. The epicutaneous induction was carried out with 0.4 mL of undiluted 2-propanol (100% w/w) at the upper left quadrant of the back of the test group animals (3 induction exposures during 3 weeks, 6 hours with webril paches under occlusion each). The challenge exposure also was conducted with 0.4 mL of undiluted 2-propanol at the lower left quadrant of the back of the test group and control group animals. Skin reactions were observed and recorded 1 hour after dermal induction and 24 and 48 hours after the challenge exposure. No positive skin reactions were observed in all animals 24 or 48 hours after challenge. Under the experimental conditions and according to the epicutaneous method of Buehler, no cutaneous reactions attributable to the sensitisation potential of 2-

propanol were observed in guinea-pigs. Therefore, the results of this study demonstrated that 2-propanol showed no evidence of contact skin sensitisation in guinea pigs

Skin sensitisation of iso propyl alcohol has been tested on guinea pigs. The test was performed under GLP according to test guidelines (OECD 406, Buehler method) and has demonstrated that the compound is not sensitizing via skin.

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4.6 Conclusion

The data related to each constituent of the product are summarized in the Table 4.

	CAS	Max % w/w	skin irritation	eye irritation	sensitization	acute systemic toxicity by inhalation	acute systemic toxicity by ingestion
Dipropylene glycol	25265-71-8	[redacted]	Slightly irritant	Slightly irritant	Non-Skin Sensitizer	Practically non-toxic	Low order of acute toxicity
Polyethylene glycol 200	25322-68-3		Not irritant	Not irritant	Non-Skin Sensitizer	Practically non-toxic	Low order of acute toxicity
Water	7732-18-5		//	//	//	//	
Ethanol	64-17-5		Not irritant	Moderately irritant	Non-Skin Sensitizer	Low order of acute toxicity	Low order of acute toxicity
Isopropanol	67-63-0		Not irritant	Irritant	Non-Skin Sensitizer	Low order of acute toxicity	Low order of acute toxicity

Table 4 - Summary of relevant toxicological data

The product could be considered slightly irritant for the skin, moderately irritant for the eye, non-skin sensitizer and practically non-toxic after acute inhalation and ingestion.
The irritation could be considered transit when the product is used according to manufacturer's instructions.

4.7 Curriculum Vitae

4.7.1 Curriculum Vitae of the Author

Work Experience:

02/2018 present	-	***Scienze Del Farmaco - Università Degli Studi Di Milano***	*Adjunct Professor* Regulatory aspects in toxicology - Legislation In European Union
08/2017 present	-	***Eurofins Biolab S.r.l.*** Vimodrone (MI) – Italy	*Senior medical devices expert* Providing consultancy services in medical device field
08/2016 07/2017	–	***Doctors with Africa CUAMM*** Cunene – Angola	*Project Administrator*
2016		***Scienze Del Farmaco - Università Degli Studi Di Milano***	*Adjunct Professor* Regulatory aspects in toxicology - Legislation In European Union
01/2014 08/2016	–	***Eurofins Biolab S.r.l.*** Vimodrone (MI) – Italy	*B.U. Medical Devices Manager* *Test Facility Manager*
2013		***Eurofins Biolab S.r.l.*** Vimodrone (MI) – Italy	*Medical Devices & Toxicology Manager* *Test Facility Manager*
2006 2012	–	***Eurofins Biolab S.r.l.*** Vimodrone (MI) – Italy	*Project Manager Medical devices* *Deputy Facility Manager* *GLP Study director*
2004 2005	–	***Barbieri s.r.l.*** Reggio Emilia – Italy	*Coordinator "Lycra Project Italy"*: technical responsible for bespoke orthesis

From November 2018, RENTIC (Registro Nazionale dei Tossicologi Italiani Certificati) member.
From June 2018, Healthcare Engineering HAS consultant for EU commission (manged by EY).
From 2009 to 2018, member of ISO TC 194, CEN TC 206 and UNI TC U4220 about biological evaluation of medical device.
From 2006 to 2018, member (chairman from 2008 to 2016) of Medical Device Technical Commission U4220 of UNI (Italian Organization for Standardization).

Professional Qualifications
2018 European Registered Toxicologist
2015 Professional Industrial Engineering qualification
Academic training
2014 *University of Surrey - Faculty of Health and Medicinal Sciences*
Master of Science in Applied Toxicology
2004 *Politecnico di Milano*
Master of Science in Biomedical Engineering

Additional Training

2018	***Eurofins***	Lean for leaders

	Università degli studi di Milano	Giornata di studio – Sperimentazione Con i dispositivi medici
	ESLAV, ECLAM, AAALAC, SECAL	ESLAV, ECLAM, AAALAC, SECAL Conference 2018 - Barcelona
	LS Academy	Clinical evaluation of Medical Device according to MDR
2010	***Eurofins|Biolab SpA***	Lab management in compliance with UNI CEI EN ISO/IEC 17025:2005
2009	***Eurofins|Biolab SpA***	Analytical Method Validation
	Agilent Technologies	Basis of HPLC
2008	***Eurofins|Biolab SpA***	Cleaning, washing and re-sterilization validation of medical device Risk management for M.D. according to EN ISO 14971: interpretation and application
2007	***Management Forum***	"Biological Evaluation Of Medical Devices" The New Approach and its Practical Application
2006	***Biolab SpA***	Sterile medical device Borderline medical device "Cleaning Validation" Clinical evaluation of medical device
2002	***XXI scuola annuale di Bioingegneria***	Tissue engineering

Articles

2018 De Jong, W.H., et al. "Round robin study to evaluate the reconstructed human epidermis (RhE) model as an in vitro skin irritation test for detection of irritant activity in medical device extracts" Toxicology in Vitro (2018), https://doi.org/10.1016/j.tiv.2018.01.001

5 Filing

All the documentation is filed in the archives of Eurofins Biolab Srl for ten years after the issuing of the final report.
At the end of the conservation period, the Sponsor may request an extension of the conservation of all or part of the products for a further period, or their restitution. A suitable agreement shall be drafted in this case.

6 Procedures

All procedures used during this study are recorded in the test facility Eurofins Biolab Srl.

7 Bibliography

- SIDS Initial Assessment Report for 11th SIAM (USA, January 23-26, 2001) Chemical Name: Dipropylene glycol, mixed isomers and dominant isomer CAS No: 25265-71-8 and 110-98-5
- https://echa.europa.eu/registration-dossier/-/registered-dossier/11848/7/3/1
- SIDS Initial Assessment Report For SIAM 19 Berlin, Germany, 19 – 22 October 2004 Chemical Name: ETHANOL
- EPA/690/R-14/009F Final 9-16-2014 Provisional Peer-Reviewed Toxicity Values for Isopropanol (CASRN 67-63-0)
- https://echa.europa.eu/it/registration-dossier/-/registered-dossier/15339/7/5/1
- https://echa.europa.eu/it/substance-information/-/substanceinfo/100.122.956
- https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2005.202

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8 Signatures

This statement was prepared based upon the information provided by the Sponsor related to substance to be evaluated and of the manufacturing process of the drug product; the author is not responsible for their correctness.
The author does not assume any liability for potential hazards or adverse clinical reactions that may be caused by the evaluated product.

Author:

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Senior Consultant

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Reviewed and electronically signed for Study Technical Supervisor Approval by
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