Journal of Pharmaceutical Analysis 2014;4(5):303–315

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Journal of Pharmaceutical Analysis

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ORIGINAL ARTICLE

Risk evaluation of impurities in topical excipients: The acetol case

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Received 19 September 2013; accepted 27 December 2013
Available online 8 January 2014

KEYWORDS

Acetol;
Impurity;
Excipients;
Transdermal penetration;
Specification limits

Abstract Pharmaceutical excipients for topical use may contain impurities, which are often neglected from a toxicity qualification viewpoint. The possible impurities in the most frequently used topical excipients were evaluated *in-silico* for their toxicity hazard. Acetol, an impurity likely present in different topical pharmaceutical excipients such as propylene glycol and glycerol, was withheld for the evaluation of its health risk after dermal exposure.

An *ex-vivo in-vitro* permeation study using human skin in a Franz Diffusion Cell set-up and GC as quantification methodology showed a significant skin penetration with an overall K_p value of 1.82×10^{-3} cm/h. Using these data, limit specifications after application of a dermal pharmaceutical product were estimated. Based on the TTC approach of Cramer class I substances, *i.e.* 1800 μg/(day•person), the toxicity-qualified specification limits of acetol in topical excipients were calculated to be 90 μg/mL and 180 μg/mL for propylene glycol and glycerol, respectively.

Abbreviations: API, Active pharmaceutical ingredient; DD, Dermal drugs; D_m, Diffusion coefficient; DP, Drug product; DS, Drug substances; FDC, Franz diffusion cells; EC, European commission; EFCG, European fine chemical group; GMP, Good manufacturing practice; ICH, International conference on harmonization; IPEC, International pharmaceutical excipient council; J_{ss}, Transdermal steady-state flux; K_m, Partitioning coefficient; K_p, permeability coefficient; PAH, Polycyclic aromatic hydrocarbon; PBS, Phosphate buffered saline; PG, Propylene glycol; QbD, Quality-by-Design; SCCS, Scientific committee on consumer safety; SE_{dermal}, Systemic exposure after dermal contact; t_{lag}, Lag time; TTC, Threshold of toxicological concern

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Peer review under responsibility of Xi′an Jiaotong University.

http://dx.doi.org/10.1016/j.jpha.2013.12.006

It is concluded that setting specification limits for impurities within a quality-by-design approach requires a case-by-case evaluation as demonstrated here with acetol.

1. Introduction

Drug products (DPs) contain active pharmaceutical ingredients (APIs) or drug substances (DSs) and excipients. These excipients or auxiliary substances, defined as "any constituent of a medicinal product that is not an active substance" [1] are intended to guarantee the required physical, microbiological, chemical and biopharmaceutical properties of the formulation. Contrary to APIs, excipients fundamentally are pharmacologically inert. Nevertheless, they actually are important actors in the final product. They can exert not only pharmaceutical functions, but also toxicological effects or might interact with active substances and hence modify the biofunctionality [2]. However, the legal quality framework for excipients is currently not so well developed as it is for APIs. Only in recent ICH Q8/Q9 and the EC directive 2011/62/EU, excipients are mentioned in a rather general and vague way. Next to the "generic" impurities like residual solvents, heavy metals or mycotoxins [3], more structurally related impurities can also be present in excipients, for which no specific detailed regulatory guidance is present [4]. Nevertheless, as the quality of excipients cannot only significantly influence the DP quality, but is also more difficult to control, it is expected that excipient quality will be further developed in the years to come. Moreover, most often no quality distinction towards impurities is made between the intended routes of administration. It seems justified that higher levels of impurities might be acceptable in certain cases such as topical application. As part of the quality risk assessment during product development, justification for these impurity levels should be made on a case-by-case basis. In this study, we will focus on the risk of related impurities present in topical excipients. Moreover, acetol, a selected possible impurity in frequently used topical excipients *i.e.* propylene glycol and glycerol, will be evaluated for its toxicity risk after dermal application.

2. Materials and methods

2.1. Impurities in topical excipients

To map the impurities present in topically applied excipients, a database of excipients used in dermal drugs was created. Applying the Handbook of Pharmaceutical excipients (4th edition) [5], an exhaustive list of pharmaceutical excipients ($n=250$) was obtained (see Table A.1). To perform a complete search of dermal drugs, the THERIAQUE® database (http://www.theriaque.org) was used (June 2009). This database contains official regulatory and validated bibliographical information about all drugs available in France, considered as a representative country in Europe. The "multi-choice search function", allowing selection of drugs on multiple criteria such as the name of the medicine or compounds (active substance or excipient) and administration route, was used. Administration way "cutaneous" OR "topical" OR "transdermal" was selected as search criterion. After elimination of products with identical composition but different packaging sizes, a total of 708 dermal drugs ($N_{DD,total}$) was obtained. For each of the 250 excipients in the pharmaceutical excipient database, the absolute number of dermal drugs wherein they occur ($N_{DD/excip}$), was tabulated (Table 1). Excipients not present in the dermal drugs ($n=133$) were excluded. Hence, 117 excipients used in preparations for cutaneous applications (dermal drugs) were retained for further analysis. From the absolute number of dermal drugs in the 117 dermally used excipients, it was calculated that in total 2499 excipients are used in the 708 dermal drugs $N_{excip,total}=\sum_{i=1}^{n=117} N_{DD/excip}$. Hence, the relative frequency of occurrence of one excipient in the total number of excipients used in dermal drugs can be calculated as follows (Table 1):

$$\text{Relative frequency}_{excip}(\%) = 100(N_{DD/excip}/N_{excip,total}) \quad (1)$$

The excipients were then ranked according to their relative frequency$_{excip}$ (from high to low) and subsequently, the cumulative frequency$_{excip}$ (%) was calculated. For the excipients covering 80% of the total use in dermal drugs, a literature-based impurity database was created. The European Pharmacopoeia (Ph. Eur.) 7th edition (2011), United States Pharmacopeia (USP) 32nd edition (2010) and International Pharmacopoeia (Ph. Int.) 4th edition (2011) as well as the search engines Web of Science, PubMed and Google (Books) were consulted (dd. October 2011). The name of the excipient and 'impurit*' were used with the Boolean operation "AND". Moreover, other possible impurities were searched for via the published industrial synthesis routes of the excipients. Finally, the *in-silico* toxicity of all found "related" impurities was examined using Derek Nexus 2.0 (Lhasa Limited) software.

2.2. Chemicals and reagents

Acetol, glycerol ($\rho=1.261$ g/mL) and absolute ethanol (EtOH) were supplied by Sigma Aldrich (Bornem, Belgium). *N*-butanol and sodium sulfate (Na_2SO_4) were purchased from Merck (Darmstadt, Germany). Ultrapure water (H_2O) was produced by an Arium 611 purification system (Sartorius, Göttingen, Germany), resulting in ultrapure water of 18.2 MΩ cm quality. Acetone was obtained from Fisher Scientific (Leicestershire, UK). Ethanol denatured with up to 5% ether was bought from Chem Lab (Zedelgem, Belgium). 0.01 M phosphate buffered saline (PBS) was purchased from Sigma (St. Louis, MO, USA). Analytical grade propylene glycol ($\rho=1.036$ g/mL) was supplied by Riedel-de Haën (Seelze, Germany).

2.3. Analytical methodology for acetol

2.3.1. Sample preparation

Aqueous receptor fluid samples were generated during the FDC experiment. To 200 µL aqueous samples, 100 µL acetone was added. About 80 mg of anhydrous Na_2SO_4 was added to capture the water. After vortexing and centrifugation, the supernatant was collected. This water-removal procedure was repeated two more times on the obtained supernatant.

Table 1 Frequency of topical excipients in dermal drugs.

#	Excipient	$N_{DD/excip}$	Relative frequency$_{excip}$ (%)
1	Water	438	17.53
2	Propylene glycol	191	7.64
3	Paraffin (=hard wax)	165	6.60
4	Ethanol (=alcohol)	146	5.84
5	Polyethylene glycol (=macrogol)	140	5.60
6	Petrolatum (=paraffin, yellow soft)	113	4.52
7	Carbomer (=acrylic acid polymer)	91	3.72
8	Glycerol	90	3.60
9	Sodium hydroxide	68	2.72
10	Triethanolamine (=trolamine)	63	2.52
11	Cetyl alcohol	62	2.48
12	Isopropyl alcohol (=isopropanol)	54	2.16
13	Cetostearyl alcohol	53	2.12
14	Lanolin (=wool fat)	49	1.96
15	Methylparaben (=methyl parahydroxybenzoate)	47	1.88
16	Butylated hydroxytoluene (=BHT)	42	1.68
17	Butylated hydroxyanisole (=BHA)	41	1.64
18	Benzyl alcohol	36	1.44
19	Propylparaben (=propyl parahydroxybenzoate)	35	1.40
20	Dimet(*h*)icone	34	1.36
21	Benzoic acid	29	1.16
22	Talc	21	0.84
23	Isopropyl miristate	20	0.80
24	Stearic acid	20	0.80
25	Hydroxypropylcellulose	19	0.76
26	Sodium laurylsulfate	19	0.76
27	Citric acid monohydrate	18	0.72
28	Poloxamer	18	0.72
29	Sorbitan	18	0.72
30	Sodium chloride	17	0.68
31	Sorbic acid (=hexadienoic acid)	17	0.68
32	Hydroxyethylcellulose	16	0.64
33	Potassium sorbate	12	0.48
34	Povidone (=polyvidone)	12	0.48
35	Phenylethyl alcohol (=benzene ethanol)	11	0.44
36	Imidurea	10	0.40
37	Hydrochloric acid (=chlorohydric acid)	10	0.40
38	Lactic acid (=2-hydroxypropanoic acid)	10	0.40
39	Sorbitol	9	0.36
40	Diethanolamide	8	0.32
41	Castor oil	7	0.28
42	Phosphoric acid	7	0.28
43	Silicone	7	0.28
44	Sodium citrate	7	0.28
45	Titanium dioxide	7	0.28
46	Xanthan gum	7	0.28
47	Acetic acid (=ethanoic acid)	6	0.24
48	Alpha tocopherol (=vitamin E)	6	0.24
49	Diethanolamine (=DEA)	6	0.24
50	Docusate sodium	6	0.24
51	Ethyl acetate	6	0.24
52	Medium-chain triglycerides	6	0.24
53	Phenoxyethanol	6	0.24
54	Chlorocresol	5	0.20
55	Peanut oil	5	0.20
56	Polyester	5	0.20
57	Sodium citrate anhydrous	5	0.20
58	Wax microcrystalline (=petroleum wax)	5	0.20
59	Acetic acid, glacial	4	0.16
60	Almond oil	4	0.16
61	Cholesterol	4	0.16
62	Ethylene glycol palmitostearate	4	0.16
63	Ethylcellulose	4	0.16

Table 1 (*continued*)

#	Excipient	$N_{DD/excip}$	Relative frequency$_{excip}$ (%)
64	Ethyl oleate	4	0.16
65	Kaolin	4	0.16
66	Menthol (=hexahydrothymol)	4	0.16
67	Polyoxyethylen	4	0.16
68	Butylparaben (=butyl parahydroxybenzoate)	3	0.12
69	Castor oil hydrogenated	3	0.12
70	Cyclomethicone (=dimethylcyclopolysiloxane)	3	0.12
71	Gelatine	3	0.12
72	Oliv oil	3	0.12
73	Sodium phosphate, dibasic	3	0.12
74	Wax white	3	0.12
75	Ascorbic acid (=vitamin C)	2	0.08
76	Benzyl benzoate	2	0.08
77	Cyclodextrin	2	0.08
78	Dimethyl	2	0.08
79	Edetic acid	2	0.08
80	Ethylparaben	2	0.08
81	Hypromellose	2	0.08
82	Isopropyl palmitate	2	0.08
83	Lactose	2	0.08
84	Magnesium silicate	2	0.08
85	Methylcellulose	2	0.08
86	Polyvinyl alcohol	2	0.08
87	Propyl gallate	2	0.08
88	Propylene carbonate (=carbonic acid)	2	0.08
89	Sodium benzoate	2	0.08
90	Sodium bicarbonate	2	0.08
91	Starch maize	2	0.08
92	Wax emulsifing non ionic (macrogol 800)	2	0.08
93	Ascorbyl palmitate (=vitamin C palmitate)	1	0.04
94	Bentonite	1	0.04
95	Benzalkonium chloride	1	0.04
96	Bronopol	1	0.04
97	Cellulose acetate	1	0.04
98	Chlorhexidine	1	0.04
99	Crospovidone	1	0.04
100	Dextrin	1	0.04
101	Ethyl vanillin	1	0.04
102	Glucose, liquid	1	0.04
103	Glyceryl behenate	1	0.04
104	Lanolin alcohol (=wool wax alcohol)	1	0.04
105	Lecithin	1	0.04
106	Lomexin	1	0.04
107	Oleic acid	1	0.04
108	Phenol (=hydroxybenzene)	1	0.04
109	Phenylmercuric nitrate	1	0.04
110	Potassium chloride	1	0.04
111	Propylen glycol alginate	1	0.04
112	Simethicone	1	0.04
113	Sodium alginate	1	0.04
114	Starch	1	0.04
115	Triacetine (=glycerol triacetate)	1	0.04
116	Triethyl citrate (=citric acid)	1	0.04
117	Vanillin	1	0.04
	SUM	$N_{excip,total}=2499$	100.00

2.3.2. GC-FID method description and verification

The acetol content in FDC experiments was assayed using a high-throughput GC-FID method. The GC apparatus consisted of a separation module provided with a Flame Ionization Detector (FID) controlled by TotalChrom (all Perkin Elmer, Massachusetts, USA). GC separations were performed using an AT™–AQUAWAX (30 m × 0.32 mm, 0.25 μm film thickness) capillary column (Grace, Berlin, Germany) maintained at 100 °C. Injector and detector temperatures were both 250 °C. Nitrogen was used as a carrier gas at a flow rate of 0.5 mL/min. The injection volume was 1 μL (split, 1:50). Total analysis time was 8 min. *N*-butanol was used as internal standard.

Method verification was performed. No interference with other sample compounds (propylene glycol, and glycerol) was observed. Linearity was assured in a working range of 14.53 µg/mL (LOQ) up to 250 µg/mL. A recovery of 94.92 ± 4.30% (mean ± RSD, $n = 3$) was found. The LOD was determined to be 4.36 µg/mL. Moreover, a precision of 1.8% ($n = 9$) at a concentration level of 100 µg/mL was observed.

2.3.3. *Franz diffusion cell experiments using human skin*

Dermal absorption data of acetol in several dose formulations were obtained using human split-thickness skin in a static FDC set-up (Logan Instruments Corp., New Jersey, USA) with a receptor compartment of 5 mL. All conditions were performed using a randomized blocked design ($n = 3$–5). Excised human skin from six female patients, who had undergone an abdominoplastic procedure, was used (49 ± 5 years old, mean ± SEM). Skin preparation was made according to the internationally accepted guidelines [6].

Immediately after the surgical removal, the skin was cleaned with 0.01 M PBS (pH 7.4) and the subcutaneous fat was removed. The skin samples were wrapped in aluminum foil and stored at −20 °C. Just before the experiments, the skin samples were thawed and dermatomed to a pre-set thickness of 400 µm. The experimentally obtained thickness of the skin, determined using a micrometer (Mitutoyo, Tokyo, Japan), was 416 ± 7 µm (mean ± SEM, $n = 66$). Skin samples were sandwiched between the donor and the receptor chambers of the diffusion cells (0.64 cm^2 diffusion area) and held together with a clamp. The receptor compartment was filled with receptor medium (*i.e.* 0.01 M PBS), making sure all air under the skin was removed. The whole assembly was fixed on a magnetic stirrer and the solution in the receptor compartment was continuously mixed using a Teflon coated magnetic stirring bar (400 rpm). Before starting the skin experiments, skin impedance was measured using an automatic micro-processor controlled LCR Impedance Bridge (Tinsley, Croydon, U.K.) in the R PAR 100 mode to ensure that there was no skin damage (skin integrity test). Skin pieces with an impedance value below 10 kΩ were discarded and replaced [7]. As no dermal exposure data are currently available for acetol, the infinite dose approach was preferred [8]. 500 µL of a 5% acetol (m/V) solution in four different vehicles was brought on the skin: propylene glycol/water (50/50, V/V), glycerol/water (50/50, V/V), ethanol/water (50/50, V/V) and pure water. Concentrations in these donor solutions were also experimentally determined by the high-throughput GC-FID method and used in the calculations. The donor compartment was then covered with Parafilm (American National Can™, Chicago, USA) and the temperature of the receptor compartment was kept at 32 ± 1 °C by a water jacket. FDC samples of the receptor fluid (200 µL) were drawn at regular time intervals from the sample port (1, 2, 4, 8, 12, 21 and 24 h) and were immediately replaced by 200 µL fresh receptor fluid solution. The analytically determined acetol assay values in the FDC samples were correspondingly corrected for the replenishments. At the end of the experiment (*i.e.* after 24 h), the skin surfaces were swabbed with cotton wool. The quantity of the remained dose was determined by the GC-FID method. The epidermis and dermis were separated with a tweezer and acetol was extracted from both skin layers with ethanol to construct a mass balance, with an overall recovered value of 116.05 ± 3.24% (mean ± SEM, $n = 23$).

2.3.4. *Kinetic analysis of Franz diffusion cell data*

The skin permeation parameters were calculated from the linear portion of the individual plots of the cumulative amount acetol permeated as a function of time. After the equilibration phase, a linear relationship of all individual curves (*e.g.* R^2 not less than 0.869) was observed. Moreover, steady-state and sink conditions were confirmed: after 24 h, only 1.3–10.0% of the dose applied was cumulatively found in the receptor chamber. For ethanol as vehicle, the last time point was not considered in further calculations. At this point, the cumulative curves flattened, indicating the loss of sink conditions.

The steady-state flux (J_{ss}) was obtained from the slope of the linear part of the curve divided by 0.64 to correct for the exposed skin area. The lag time (t_{lag}) was estimated by extrapolating the linear portion of the curve to the time-axis. The maximal absorption ($Q_{max,24\,h}$), expressed as percentage of the applied dose, was calculated as the sum of the acetol quantity found in the receptor fluid, epidermis and dermis after 24 h. From these three experimentally secondary kinetic parameters, the apparent primary transdermal parameters, *i.e.*, the permeability coefficient K_p, the diffusion coefficient D_m and skin/dose-vehicle partitioning coefficient K_m, were calculated as follows [9]:

$$K_p = J_{ss}/C_v \tag{2}$$

where C_v is the experimentally determined concentration of acetol in the dose formulation.

$$D_m = d^2/(6t_{lag}) \tag{3}$$

$$K_m = (K_p d)/D_m \tag{4}$$

where d is the measured tissue thickness.

To obtain the mean transdermal permeation parameters, the individual values for each replication were averaged for the four investigated conditions.

3. Results

3.1. *Impurities in topical excipients*

A pharmaceutical excipient list was constructed (Table A.1) and explored to obtain information about the absolute and relative frequency of occurrence of these excipients in dermal drugs (Table 1). From these data on, the cumulative frequency of dermal excipients was calculated (Fig. 1). A large majority of dermal drugs were composed of only a few different excipients. Indeed, only 21 of the 117 excipients used in the dermal drugs (*i.e.* 18%) represented 80% of the total use. Although water is the most abundant excipient in dermal drugs, it will be excluded for further investigation. For the remaining 20 most important excipients, all impurities found in the pharmacopoeias, literature [10–29] and from typical synthesis pathways are listed in Table 2.

Apart from the "generic" impurities like residual solvents, heavy metals, pesticides, and mycotoxins [3,8], which were not the subject of this investigation, the more structurally related impurities were considered ($n = 115$, presented in italic in Table 2) and *in-silico* evaluated on their intrinsic toxicity. It is interesting to note that the different consulted pharmacopoeias sometimes indicate not only different specification limits for specified impurities, but even different impurities. Moreover, the pharmacopoeias and literature information also include impurity-classes, *e.g.* PAHs in paraffin, petrolatum and pesticide residues in lanolin and organic

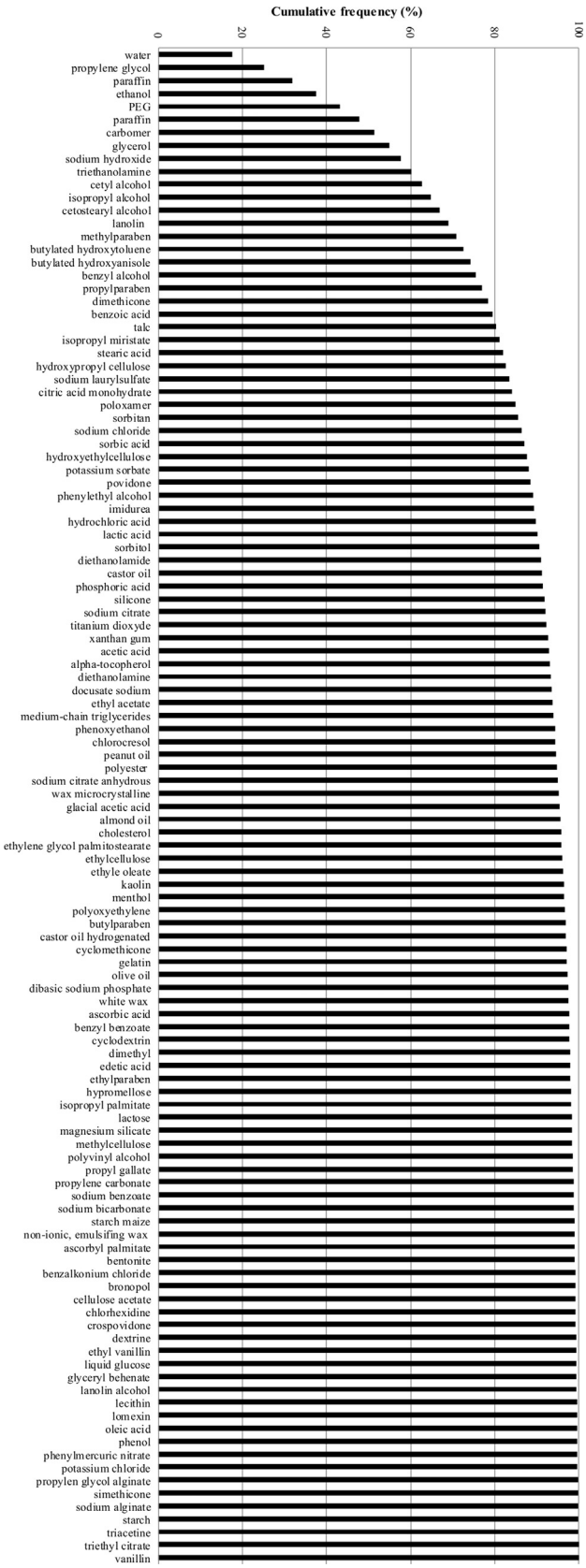

Fig. 1 Cumulative frequency (%) of excipients in topical drugs.

hydroperoxides in polyethylene glycol. These specified, individually-unidentified impurities were searched in the literature: phenanthrene was the only reported PAH impurity in petrolatum [20], while for paraffin, phenanthrene [15], naphthalene [13], benzo[b]fluoranthene and benzo[a]pyrene [14] have been specified. As paraffin and petrolatum are both purified from crude oil, both excipients are expected to contain the same PAH impurities. Therefore, all these four PAHs will be included in the toxicity evaluation. On the contrary, the exact structures of organic hydroperoxide impurities were not further identified in the literature. Hence, 1-hydroperoxyethane-1,2-diol will be taken as the model compound for the *in-silico* toxicity evaluation of this class of impurities. Fig. 2 presents the *in-silico* (Derek Nexus) toxicity information for all 115 impurities over the different toxicity endpoints, subdivided in mammals or bacteria. Approximately half ($n=23$) of the 48 endpoints investigated by Derek Nexus gave an alert for the topical excipient impurities. Possible alerts for the endpoints are displayed with the level of likelihood, a qualitative indication for the toxicity prediction of a chemical (*i.e.* certain, probable, plausible, equivocal, doubted, improbable, and impossible), which is determined by structure-activity relationships ((Q)SARs) and other expert knowledge rules. Empty cells in the matrix of Fig. 2 indicate that no structural alerts were found in the compound for the corresponding endpoint (*e.g.* carcinogenicity in butan-1-ol). This implies that there is no evidence of toxicity, nor evidence of non-toxicity.

3.2. *Toxicity priority list of the impurities found in topical excipients and choice of acetol*

A toxicity priority list (Table 3) of the investigated impurities was derived from the *in-silico* toxicity outcome in Fig. 2.

Toxicity class "$\geq$ probable" represents impurities having a level of likelihood of "probable" for at least one endpoint, class "plausible" encloses impurities having a level of likelihood of "plausible" for at least one endpoint and class "$\leq$ equivocal" contains impurities with a level of likelihood of "equivocal" or lower.

The toxicity class "$\geq$ probable" encompasses impurities for which the risk after dermal exposure has already been investigated, *i.e.* PAHs [30], propylene oxide [31], acetaldehyde [32], formaldehyde [33], benzene [34], furfural [35], methanol [36], acrolein [37], dihydroxyacetone [38], phenol [39], hydroquinone [40], *p*-methoxyphenol [41], *p*-cresol [42] and phthalic anhydride [43]. Due to the chemical and physical instability of allylchloride [44], and hazardous gasses like ethylene oxide and benzoyl chloride, their presence in topical formulations contacting the skin is highly questionable. Moreover, dermal assessment data are available for ethylene oxide [45,46]. The second class of impurities contains a variety of compounds with "plausible" toxicity. Considering the relative frequency of occurrence of excipients in the dermal drugs, acetol (1-hydroxypropan-2-one or 1-hydroxyacetone) was found to be the most important impurity. This hydrophilic low mass molecule (MW = 74 g/mol, log $P_{ow} = -0.78$) has been reported in two excipients *i.e.* propylene glycol and glycerol. After water, propylene glycol is the most abundantly present topical excipient and together with glycerol, they represent 11% of excipients use in topical drugs. Exposure to other impurities from this second

Table 2 Impurities of the most common topical excipients

Excipient	Ph. Eur. (limit)	USP (limit)	Ph. Int. (limit)	Literature	Other possible impurities
Propylene glycol	Heavy metals (5 µg/mL); sulfated ash (0.01%)	Heavy metals (5 µg/mL); chloride (0.007%); sulfate (0.006%)	Heavy metals (5 µg/mL); sulfated ash (0.01%)	*Acetol* [10]; *dipropylene glycol* [11]; *tripropylene glycol* [11]; *diethylene glycol* [12]	*Alpha-phenylethanol, propylene; oxide; ethylbenzene; styrene; ethylbenzene hydroperoxide*
Paraffin	*PAHs*; sulfates (150 µg/mL); sulfated ash (0.05%)	*PAHs*; sulfur compounds	Sulfated ash (0.1%)	*Naphthalene* [13];*benzo[a]pyrene* [14]; *benzo [b]fluoranthene* [14]; *phenanthrene* [15]	n/a
Polyethylene glycol	*Formaldehyde* (30 µg/mL); *ethylene glycol; diethylene glycol; ethylene oxide* (1 µg/mL); *1,4-dioxan* (10 µg/mL); sulfated ash (0.20 %); heavy metals (20 µg/mL)	*Ethylene oxide* (10 µg/mL); *1,4-dioxan* (10 µg/mL); *ethylene glycol; diethylene glycol*	*Diethylene glycol;* heavy metals (50 µg/mL); sulfated ash (1%)	*Acetaldehyde* [12]; *formaldehyde*; *formic acid* [16]; *organic hydroperoxide* [17]; *free ethylene oxide*; *1,4-dioxane*; *ethylene glycol; diethylene glycol* [18]	n/a
Ethanol	*Acetal; acetaldehyde; acetone; benzene* (2 µg/mL); *cyclohexane; methanol* (100 µg/mL); *methyl ethyl ketone (butan-2-one); methyl isobutyl ketone; propan-1-ol; isopropanol (propan-2-ol); butan-1-ol; butan-2-ol; 2-methylpropanol (isobutanol); furfural; 2-methylpropan-2-ol; 2-methylbutan-2-ol; pentan-2-ol; pentan-1-ol; hexan-1-ol; heptan-2-ol; hexan-2-ol; hexan-3-ol*	*Methanol* (100 µg/mL); *acetal; acetaldehyde; benzene* (2 µg/mL)	*Aldehydes methanol benzene*	*Acetaldehyde*; *isobutyl acetate; diethylacetal; crotonaldehyde; 1,1-diethoxypentane* [19]	n/a
Petrolatum	*PAH;* sulfated ash (0.05%)	n/a	Sulfated ash (0.10%)	*Phenanthrene derivates* [20]	n/a
Carbomer	*Free acrylic acid* (0.25%); *benzene* (2 µg/mL); sulfated ash (4%); heavy metals (20 µg/mL)	*Acrylic acid* (0.25%); *benzene*[a](2 µg/mL); heavy metals (20 µg/mL); ethyl acetate[c] (0.5%); cyclohexane[b] (0.3%)	Sulfated ash (0.10%)	*Furfural* [21]	*Acetaldehyde; formaldehyde; acrolein; methylfuran; furfural*
Glycerol	*Diethylene glycol* (0.05%); *ethylene glycol; propylene glycol; aldehydes* (10 µg/mL); esters; halogenated compounds (35 µg/mL); sugars; chlorides (10 µg/mL); sulfated ash (0.01%); heavy metals (5 µg/mL)	Chloride (0.001%); sulfate (0.002%); heavy metals (5 µg/mL); chlorinated compounds (0.003%); fatty acids; esters; *diethylene glycol* (0.025%); *ethylene glycol* (0.025%)	Heavy metals (5 µg/mL); chlorides (10 µg/mL); sulfates (20 µg/mL); sulfated ash (0.10%); water (2%); chlorinated compounds; fatty acids and esters; *aldehydes and reducing substances*	*Acetol* [10]; *glyceraldehyde* [22]; *dihydroxyacetone* [22]; *acrolein* [23]	*Dichlorohydrin; epichlorohydrin;, 1,3-propanediol; propylene glycol; allylchloride; di- and tripropylene glycol; propene*
Sodium hydroxide	Carbonates (2%); chlorides (50 µg/mL); sulfates (50 µg/mL); iron (10 µg/mL); heavy metals (20 µg/mL)	Potassium; heavy metals (0.003%); sodium carbonate (3%)	Heavy metals (10 µg/mL); arsenic (4 µg/mL); aluminum, iron and insoluble matter in HCl; potassium; sulfates (1200 µg/mL); chlorides (700 µg/mL)	n/a	n/a
Trolamine	*Ethanolamine* (0.10%); *diethanolamine* (0.50%); *N-nitrosodiethanolamine* (24 ng/mL); sulfated ash (0.10%); heavy metals (10 µg/mL)	n/a	n/a	n/a	n/a

Table 2 (*continued*)

Excipient	Ph. Eur. (limit)	USP (limit)	Ph. Int. (limit)	Literature	Other possible impurities
Cetyl alcohol	n/a	n/a	Sulfated ash (0.1%); paraffin	*Stearyl alcohol* [24]; *myristyl alcohol* [24]; *lauryl alcohol* [24]	n/a
Isopropyl alcohol	*Acetone;* benzene (1 µg/mL); *di-isopropyl ether; diethylether; methanol; propan-1-ol*	*Diethylether; di-isopropylether; acetone; butan-2-ol*	*Aldehydes* and ketones	n/a	n/a
Cetostearyl alcohol	n/a	n/a	Paraffin	*Octa-, nonadecane; eicosane, henicosane; 1-do-,tri-, tetra-, penta-, hexa-; heptadecanol; 2-tetra-, penta-, hexa-, hepta-, octa-, nonadecanol; 3-tetra-, penta-, hexa-, hepta-, octa-, nonadecanol; do-, tri-, tetra-, penta-, hexa-, hepta-, octadecanal* [25]	n/a
Lanolin	BHT (200 µg/mL); paraffin (1%); pesticides residues (1 µg/mL); chlorides (150 µg/mL); sulfated ash (0.15%)	*Chloride* (0.035%); ammonia; pesticide residues (40 µg/mL); petrolatum	Paraffin; ammonia	*Chlorfenvinphos*; cypermethrin; diazinon [26]	n/a
Methyl paraben	*4-Hydroxybenzoic acid* (0.50%); *ethylparaben; propylparaben; butylparaben;* sulfated ash (0.10%)	n/a	Sulfated ash (0.10%)	n/a	*Phenol*
Benzyl alcohol	*Benzaldehyde* (0.15%); *cyclohexylmethanol* (0.10%)	*Cyclohexylmethanol; benzaldehyde*	Chlorinated compounds; aldehydes; sulfated ash (0.05%)	*Benzaldehyde dibenzylacetal* [27]; *dibenzyl ether* [28]	*Benzylchloride; dibenzylether*
BHA	*Hydroquinone* (0.20%); heavy metals (10 µg/mL)	Heavy metals (0.001%)	*Hydroquinone;* sulfated ash (0.10%)	n/a	*Paramethoxyphenol; isobutene*
BHT	Sulfated ash (0.10%)	Heavy metals (0.001%)	*Hydroquinone;* sulfated ash (0.10%)	n/a	*p-Cresol, isobutene*
Propyl paraben	*4-Hydroxybenzoic acid* (0.50%); *methylparaben* (0.50%); *ethylparaben* (0.50%); *butylparaben* (0.50%); sulfated ash (0.10%)	n/a	Sulfated ash (0.10%)	n/a	*Phenol*
Dimethicone	Heavy metals (5 µg/mL)	Heavy metals (5 µg/mL); bacterial endotoxins (10 endotox u/mL)	n/a	n/a	*Chlorotrimethylsilane; dichlorodimethylsilane*
Benzoic acid	Sulfated ash (0.10%)	Heavy metals (10 µg/mL)	Heavy metals (20 µg/mL)	*Phthalic acid* [29]	*Toluene; benzoylchloride; benzotrichloride; phthalic anhydride*

Ph. Eur.:European Pharmacopoeia, 7th ed. European Directorate for the Quality of Medicines and Healthcare. Strassbourg, France, 2010–2013.
USP: United States Pharmacopeia, USP 35/NF30. The United States Pharmacopeial Convention. Rockville, MD, USA, 2012.
Ph. Int.: International Pharmacopoeia. WHO. Genève, Switzerland, 2012.
n/a: not available.
Related impurities are presented in italic.
[a]Residual solvent class 1.
[b]Residual solvent class 2.
[c]Residual solvent class 3. n/a: not available.

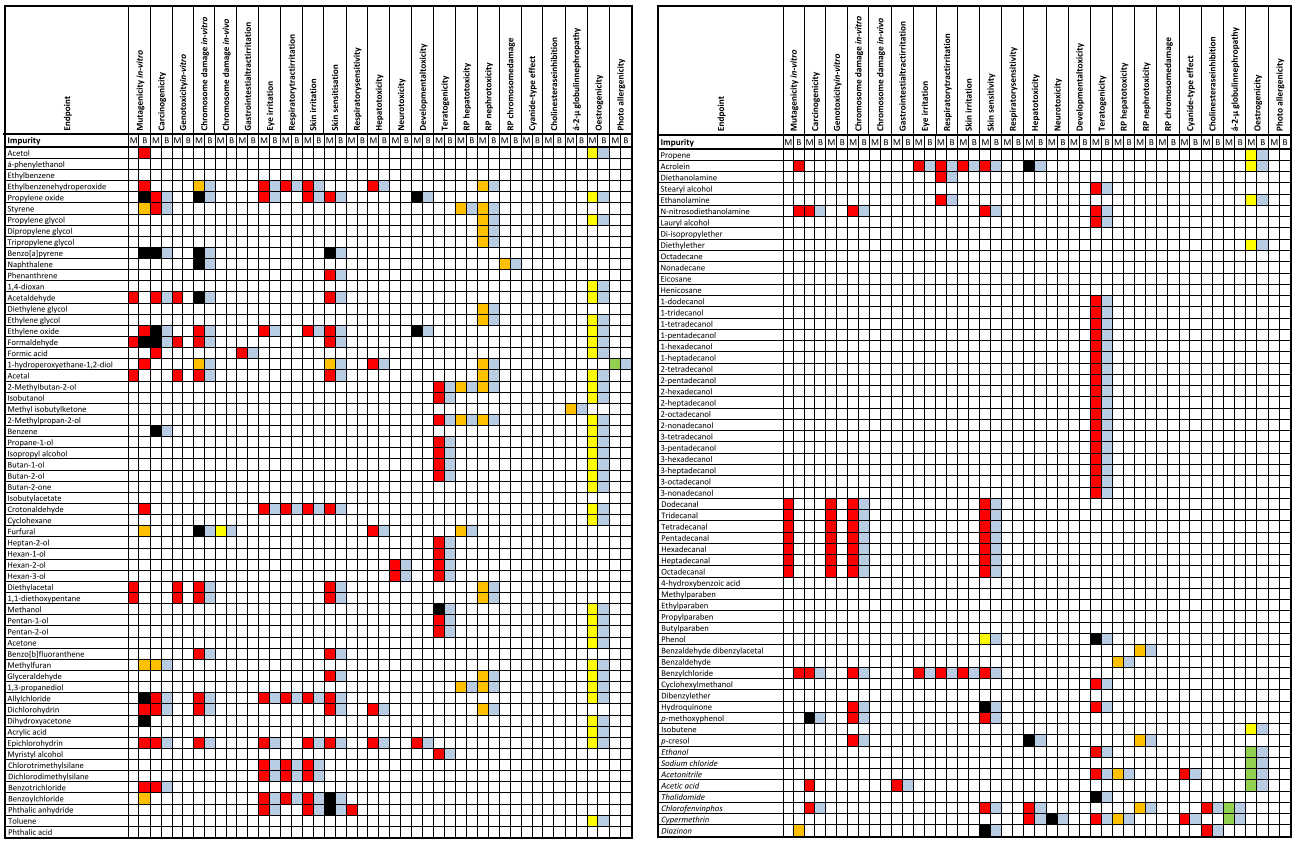

Fig. 2 Toxicity database of related impurities in topical excipients. Legend: Probable; Plausible; Equivocal; Doubted; improbable; Impossible. Species: Mammal (M); Bacteria (B). Control compounds are given in italic.

toxicity class "plausible", which are present in more than one excipient (*e.g.* phenol), occurs at a much lower relative frequency$_{excip}$ (approximately 5%). Moreover, dermal absorption data of acetol are lacking, emphasizing the need to investigate acetol.

3.3. *Dermal study using human skin*

Quantitative transdermal parameters for acetol were obtained using human skin (gold standard). Two examined vehicles, propylene glycol/water (50/50, V/V) and glycerol/water (50/50, V/V), are representative in-use conditions of dermal products. To implement the K_p sensitivity towards the choice of the solvent, 5% acetol in water and in ethanol/water (50/50, V/V) were also incorporated in the experimental design. Fig. 3 shows typical plots of the cumulative amount penetrated through the skin (µg) *versus* time (*h*) for each of the investigated formulations. Their mean transdermal parameters are given in Table 4.

The steady-state apparent skin permeability of acetol in ethanol/water (50/50, V/V) ($K_p = 3.91 \times 10^{-3}$ cm/h) was significantly higher than in the other vehicles. This is not unexpected as ethanol is a well-known penetration enhancer, decreasing the barrier resistance by *i.a.* dissolving SC lipids [47]. The permeability coefficients of acetol in the other dose vehicles, also reported to be penetration enhancers [47–49], were more comparable ($K_{p,water\ and\ PG} = 1.30 \times 10^{-3}$ cm/h > $K_{p,glycerol} = 7.78 \times 10^{-4}$ cm/h).

4. Discussion

Dermal drug excipients are mostly used in much higher quantities than APIs, contrasting other dosage forms like oral tablets. However, unlike APIs and packaging material, the formal regulations on pharmaceutical excipients are rather limited. In recent ICH Q8/Q9 and EC directive 2011/62/EU, excipients are mentioned rather vague, requiring the holders of the manufacturing authorization to ensure the suitability of excipients in medicinal products. Although European authorities recognize the need for formal guidelines with draft proposals being issued, the addition of more specific GMPs for some categories of excipients (among them propylene glycol and glycerol) was drawn back. Nevertheless, some authorities are in the process of assuring the excipient quality system in one or another way [50]. Lacking an explicit formal excipient control, IPEC and partner organizations like EFCG are developing their own voluntary GMP guidelines [51]. Moreover, major pharmaceutical companies are aware of quality issues related to the use of topical excipients, and are currently tackling these [52].

Therefore, we first established a list of all excipients used in preparations for cutaneous applications, and focused on the 20 most important excipients. Next, we looked at all possible (related) impurities of these 20 excipients, and evaluated their toxicity by an *in-silico* approach. From the toxicity priority list, the impurity with the highest toxicity concern for which no dermal exposure data are available, *i.e.* acetol, was selected in order to assess its risk when dermally applied in relevant dermal drug excipients (*i.e.* propylene glycol and glycerol). Although the *in-vitro* mutagenic properties of acetol were demonstrated with positive Ames tests [53–56] and with *in-silico* Derek Nexus evaluation, EFSA reported it is not likely for acetol to be genotoxic *in-vivo* [57]. Acetol is thus classified as Cramer structural class I [57,58], with a TTC of 1800 µg/(day•person)

Table 3 *In-silico* toxicity impurity list of dermal excipients.

Class	Impurities	#
≥ **Probable**	Propylene oxide; benzo[a]pyrene; naphthalene; acetaldehyde; ethylene oxide; formaldehyde; benzene; furfural; methanol; acrolein; allylchloride; dihydroxyacetone; phenol; hydroquinone; *p*-methoxyphenol; *p*-cresol; benzoylchloride; phthalic anhydride	19
Plausible	Acetol; ethylbenzene hydroperoxide; styrene; phenanthrene; formic acid; 1-hydroperoxyethane-1,2-diol; acetal; 2-methylbutan-2-ol; 2-methylpropan-1-ol (isobutanol); 2-methylpropan-2-ol; propan-1-ol; propan-2-ol (isopropyl alcohol); butan-1-ol; butan-2-ol; crotonaldehyde; heptan-2-ol; hexan-1-ol; hexan-2-ol; hexan-3-ol; diethylacetal; 1,1-diethoxypentane; pentan-1-ol; pentan-2-ol; benzo[b]fluoranthene; glyceraldehyde; dichlorohydrin; epichlorohydrin; diethanolamine; ethanolamine; N-nitrosodiethanolamine; laurylalcohol; myristylalcohol; stearylalcohol; 1-dodecanol; 1-tridecanol; 1-tetradecanol; 1-pentadecanol; 1-hexadecanol; 1-heptadecanol; 2-tetradecanol; 2-pentadecanol; 2-hexadecanol; 2-heptadecanol; 2-octadecanol; 2-nonadecanol; 3-tetradecanol; 3-pentadecanol; 3-hexadecanol; 3-heptadecanol; 3-octadecanol; 3-nonadecanol; dodecanal; tridecanal; tetradecanal; pentadecanal; hexadecanal; heptadecanal; octadecanal; benzylchloride; cyclohexylmethanol; chlorotrimethylsilane; dichlorodimethylsilane; benzotrichloride	62
≤ **Equivocal**	Alpha-phenylethanol; ethylbenzene; propylene glycol: dipropylene glycol; tripropylene glycol; 1,4-dioxan; diethylene glycol; ethylene glycol; methyl isobutyl ketone; methyl ethyl ketone (butan-2-one); cyclohexane; isobutyle acetate; acetone; acrylic acid; methylfuran; 1,3-propanediol; propene; diisopropylether; diethylether; octadecane; nonadecane; eicosane; henicosane; 4-hydroxybenzoic acid; ethylparaben; propylparaben; methylparaben; butylparaben; benzaldehyde dibenzyl acetal; benzaldehyde; dibenzylether; isobutene; toluene; phthalic acid	34

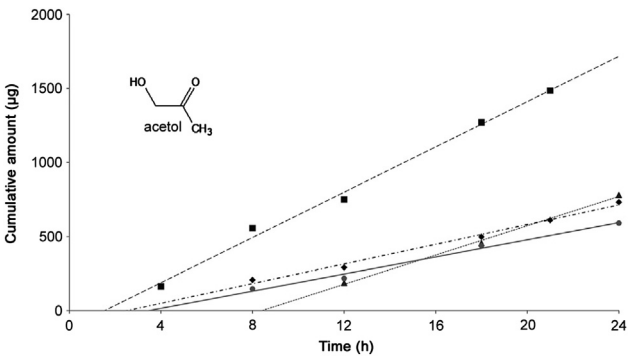

Fig. 3 Typical flux curves of acetol in the investigated vehicles. Legend: (◆) pure water, (■) ethanol/water (50/50, V/V), (▲) propylene glycol/water (50/50, V/V), and (●) glycerol/water (50/50, V/V).

[59]. The estimated food exposure of acetol as food flavoring substance [FL-no 07.169], *i.e.* 0.22 µg/(day•person), does not exceed this class I threshold and hence the EFSA panel concluded that acetol at its current use in food is safe.

Originally, the TTC approach was only used for the safety assessment of flavoring substances, but has recently been extended towards the risk assessment of impurities in therapeutic drugs and cosmetic ingredients [60]. It was concluded that the existing oral TTC values can also be used for dermal exposure, provided that some route-to-route extrapolations are considered, principally to cover the difference in bioavailability. In this study, the bioavailability after dermal exposure to acetol was addressed by performing an *ex-vivo in-vitro* transdermal experiment. Additionally, the bioavailability depends on the metabolization in the skin. Acetol is metabolized to endogenous compounds (methylglyoxal, pyruvate and glucose) in the methylglyoxal pathway [57]. All involved enzymes (CYP2E1, glutathione-S-transferase, alcohol dehydrogenase and aldehyde dehydrogenase) are also present in the skin, but their activities are lower than in other tissues [61,62]. Moreover, these enzymes are largely inactivated in once-frozen excised skin [6,63]. A slight overestimation of unmetabolized acetol reaching the systemic circulation after dermal exposure can thus be expected in our experiments.

As acetol is an impurity in the frequently used topical excipients propylene glycol and glycerol, limit specifications in these excipients were set by comparing the TTC of 1800 µg/(day•person) with the systemic exposure as defined by the Office of Solid Waste and Emergency Response (OSWER) equation [64]:

$$\mathrm{DDE} = ([\mathrm{acetol}]K_p \mathrm{SA\ ED\ EF\ EV}\ t_{event})/(\mathrm{BW\ AT}) \quad (5)$$

DDE is the systemic exposure to acetol after dermal drug application under pre-defined circumstances. To minimize the risk of acetol, this value may thus not exceed the TTC-value of 1800 µg/(day•person). The acetol exposure concentration in a dermally applied drug, [acetol], can be split up in the concentration of acetol in the excipient (*i.e.* specification limit) and the concentration of this excipient (propylene glycol or glycerol) in the dermal formulation. For information towards the latter, the THERIAQUE® database was consulted. The concentration (%, m/m) of excipient in the dermal drug formulation covering 65% of the total population was considered as an appropriate estimate in our limit specification calculations. Concentrations of propylene glycol and glycerol in dermal formulations were in this way estimated to be 10% (m/m) and 5% (m/m), respectively (Fig. 4).

The apparent permeability coefficient K_p for acetol was obtained from the *in-vitro* transdermal FDC experiment. The overall K_p (mean+1SD) of the different formulations, *i.e.* acetol in water, in 50% EtOH, in 50% PG and 50% glycerol, was calculated to be 2.5×10^{-3} cm/h. This value is intended to be an averaged estimate. The head, arms and hands were considered as the treated skin area (SA), which corresponds to 0.4991 m^2 (mean+1SD) for an adult man [65]. The exposure duration (years) (ED) for non-carcinogenic chemical exposure is equivalent to the averaging time (days) (AT) *i.e.* 25 years (9125 days). For a (worst case) chronic treatment, applied once a day (event frequency (EV) = 1 event/day), the exposure frequency (EF) was supposed to be 365 days/year. The event duration (t_{event}) was estimated to be 16 h/event, as a person is assumed to apply the formulation in the morning and will rinse it off before sleeping. Hence, using the DDE formula

Table 4 Transdermal parameters for acetol in the different vehicles (mean ± RSD (%)).

Condition	Observed secondary parameters			Apparent primary parameters		
	J_{ss} (μg/cm^2/h)	$Q_{max,24\,h}$	Lag time (h)	K_p (10^{-3} cm/h)	D_m (10^{-4} cm^2/h)	K_m
H_2O	68.3 ± 21.2	3.37 ± 11.7	2.95 ± 71.0	1.30 ± 21.2	1.28 ± 49.6	0.55 ± 77.2
50% EtOH	170.0 ± 45.1	7.28 ± 29.0	2.14 ± 51.8	3.91 ± 45.1	1.49 ± 41.8	1.19 ± 48.0
50% PG	84.6 ± 79.0	2.94 ± 76.0	5.65 ± 70.9	1.30 ± 79.0	1.07 ± 103.0	1.24 ± 91.5
50% Glycerol	58.3 ± 39.9	1.75 ± 39.0	4.39 ± 28.7	0.78 ± 40.3	0.73 ± 22.4	0.50 ± 61.4

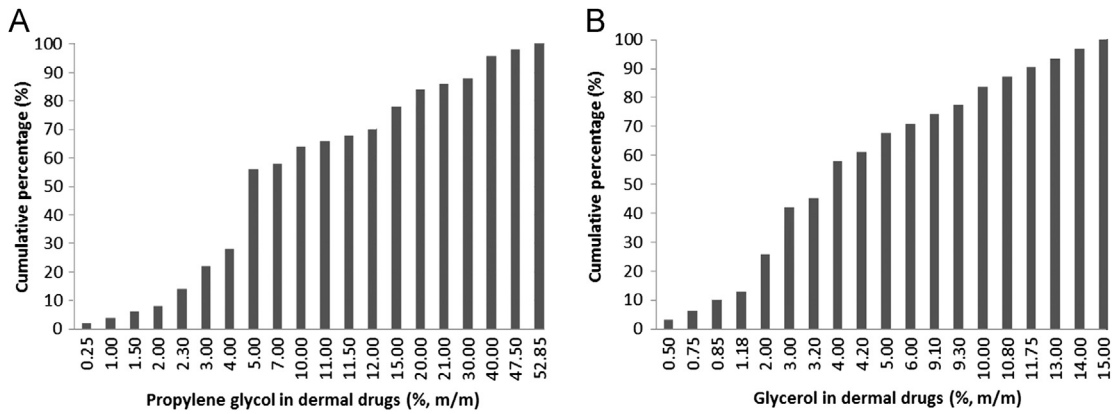

Fig. 4 Cumulative frequency (%) of (A) propylene glycol and (B) glycerol in dermal drugs.

and setting DDE = 1800 μg/(day person), the specification limits for acetol in propylene glycol and glycerol were calculated to be 90 μg/mL (parts per million) and 180 μg/mL, respectively.

HPLC–UV analysis of acetol in propylene glycol and glycerol market samples gave values ranging between 3 and 80 μg/mL [10], indicating compliance with the proposed dermal specification limits. Of course, the calculated specification limits are only applicable for dermal exposure to acetol. Assuming a bioavailability of 100% after oral intake, more strict limits are expected to be set. Although most quality regulatory guidelines still do not make a distinction between the applied routes of administration, it is justified to apply other specification limits depending on the intended administration route. Moreover, in a QbD approach, a fixed limit can be avoided by consulting a suitable design space (*e.g.* concentration of acetol in excipient × concentration of excipient in the formulation). In our approach, we assumed that only one of both excipients (propylene glycol or glycerol) is present in the dermal drug formulation. If both excipients are present in the formulation, the overall dermal specification limit (in propylene glycol plus glycerol) obviously needs to be lowered. Moreover, the dermal formulation in which the impurity occurs influences the K_p and hence the systemic exposure. For each exposure condition to a specific dermal formulation *e.g.* in the case when other components might be designed to improve drug absorption, deviating specification limits can be expected. As mentioned before, propylene glycol and glycerol are penetration enhancers themselves, but very often other penetration enhancing excipients, like ethanol, or penetration enhancing bioactive compounds, like spilanthol [66,67], are present in the topical drug formulation. Different models and literature describe the skin permeation influence of accompanying compounds [68–70], but further elaboration of their impacts is beyond the scope of this work.

Summarized, in this study, a risk priority list of related impurities in excipients for topical use was made. This central tool can be applied to judge the safety of pharmaceutical excipients. Moreover, it emphasizes that a more strict regulatory quality control for excipients is justified and that a case-by-case evaluation is required. In a study with acetol as important impurity in propylene glycol and glycerol, we introduce a general approach that can serve as risk assessment GMP guideline for hazardous impurities in dermally used excipients. It allows to set quantitative specification limits, which can be used in a first step to manage the possible risk of uncontrolled pharmaceutical excipients.

Acknowledgments

This work was funded by the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen) to Jente Boonen (091257) and by the Special Research Fund (BOF) of Ghent University to Lien Taevernier (01D23812). The authors would like to thank Remy Duvillard, Lauriane Meunier and Andy Vandamme for their technical assistance and Sian Ives for his comments on the *in-silico* toxicity section.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jpha.2013.12.006.

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