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Information for the package leaflet regarding polysorbates used as excipients in medicinal products for human use

Draft

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Information for the package leaflet regarding polysorbates used as excipients in medicinal products for human use

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Executive summary

This document has been written in the context of the revision of the Annex of the European Commission Guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' (Annex, 2017; EC, 2018). Polysorbates are currently not listed in the Annex to the guideline on 'Excipients in the label and package leaflet of medicinal products for human use'

Polysorbate 80 (PS 80, polyoxyethylene sorbitan monooleate, also known as Tween 80) and 20 (PS 20, polyoxyethylene sorbitan monolaureate, also known as Tween 20) are mixtures of the partial esters of sorbitol and its mono- and dianhydrides with oleic or lauric acid, resp., and condensed with approximately 20 moles of ethylene oxide per mole of sorbitol and its anhydrides. They are used as nonionic surfactants and as emulsifiers, being the most common surfactants used in biological medicinal products for protein stabilisation.

Polysorbate was proposed to be added on the list of excipients for which safety issues (e.g. potential cardiotoxicity, phthalate extraction from polyvinyl chloride (PVC) materials, etc.) should be considered for inclusion in the guideline.

Acute oral toxicity is low which is probably attributed to the very low oral bioavailability of intact polysorbates. The acceptable daily intake (group ADI) for polysorbates as food additives (polysorbates 20, 80, 40, 60 and 65; E 432, E 433, E 434, E 435 and E 436, respectively) was set to 25 mg/kg body weight/day by EFSA in 2015 [23].

In view of the estimated maximum oral dose of PS 80 or PS 20 in authorised medicinal products of about 1 mg/kg/day the oral exposure of PS 80 by oral formulations is estimated to be far below ADI. Therefore, a warning on the effects of polysorbates as excipients by oral administration is not considered meaningful. However, as it is known that polysorbate 80 increases gastrointestinal absorption of other drugs, this potential PK interaction should be taken into account in SmPC/PIL (see table for the package leaflet).

In contrast to the oral route, after intravenous administration (IV) the whole amount of intact polysorbates enter the bloodstream. The ability of polysorbates to enhance the uptake of drugs into the brain constitutes a potential interaction with drug substances which should be taken into account during benefit-risk evaluation of current and new parenteral products containing polysorbates. As hypersensitivity reactions including anaphylactoid shock have been observed after IV administration, a warning of allergic reactions at threshold zero is proposed.

A significant hemodynamic effect (short duration vasoplegia, left ventricular systolic pressure decreased) was observed in human adults after amiodarone IV bolus injection (Cordarone®) containing 10 mg/kg PS 80 compared to a formulation without polysorbate and benzyl alcohol. In dogs, bolus doses ≥ 10 mg/kg of PS 80 alone lead to depression of the cardiac conduction and hypotension. Thus from the totality of preclinical and clinical data a threshold of 10 mg/kg (given as bolus dose) is considered justified to trigger a warning regarding cardiovascular effects (e.g. hypotension). A small PK and safety study with anidulafungin infusions in infants and neonates with maximum PS 80 exposure of 7.7 mg/kg/day (max infusion rate over 60 min: 0.13 mg/kg/min) gives support that short term exposure of PS 80 < 10 mg/kg per day is safe even in infants and neonates.

The cardiovascular effects appeared to be rather related to the infusion rate than to the cumulative dose. This might also explain the apparent safe use of MVI paediatrics (Multi-Vitamins for Infusion), a US vitamin product for 24h infusion resulting in relatively high cumulative PS 80 exposure (32.5 mg/kg/day in 1 kg neonates), but at a quite low infusion rate of 0.023 mg/kg/min. A small PK and

safety study with anidulafungin infusions in infants and neonates with maximum PS 80 exposure of 7.7 mg/kg/day (max infusion rate over 60 min: 0.13 mg/kg/min) gives support that short term exposure at low infusion rates of PS 80 < 10 mg/kg per day is safe even in infants and neonates.

Thus, a general recommendation for risk minimisation by lowering the rate of injection/infusion is given as a comment for consideration in the SmPC of parenteral products.

Risk for a cardiotoxic/torsadogenic potential of polysorbates is supported by in vitro data on hERG current inhibition as well as from preclinical data showing an increase in effective refractory period (ERP) in guinea-pig cardiac preparations and in vivo in dogs. There is no evidence so far for depression of cardiac conduction from clinical data in humans which would allow derivation of a safety threshold for cardiotoxicity. It is concluded that further (pre-clinical and) clinical electrophysiological studies are warranted to investigate the torsadogenic potential of polysorbate 80 in detail. Currently at least a warning on the risk of concomitant use of medications that prolong the QT/QTc interval should be considered for the SmPC of all products containing polysorbates above this threshold of 10 mg/kg/day when given as bolus.

The hepatotoxic potential of polysorbates gained notoriety after the E-ferol tragedy in the 1980s when 38 infant deaths were reported after IV infusion of this Vitamin E formulation containing a mixture of polysorbate 80 (9%) and polysorbate 20 (1%) as solubilising agents. A clear dose-response relationship was found with an increased risk for severe hepatotoxicity in premature infants at a PS dose of > 80 mg/kg/d. Data suggested that the cumulative doses over 6-45 days rather than short term peak exposure levels appeared to be relevant for hepatotoxicity.

However, case reports in adults at exposures below 80 mg/kg/d may indicate an earlier onset of signs of hepatotoxicity: 35-40 mg/kg were calculated as the cumulative PS dose within 24 h identified in case reports of hepatotoxicity in adults after Amiodarone IV, e.g. showing abrupt elevation of liver enzymes. Such case reports are confounded by the fact that amiodarone itself is a hepatotoxic agent, however, the observation that subsequent oral amiodarone administrations in patients did not result in additional liver toxicity supports the association with the intravenous exposure of the excipient.

In conclusion, a threshold of 35 mg/kg/d for all age groups is suggested to trigger a warning for elevation of liver enzymes.

Polysorbates exposure via administration of therapeutic proteins and vaccines is very low (< 0.25 mg/kg) being below all thresholds apart from zero. This is considered appropriate as it is in line with the absence of any signal of cardiotoxicity or hepatatoxicity after vaccine exposure from epidemiology or pharmacovigilance.

117 **Proposal for new information in the package leaflet**

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Polysorbates **(E 432–436)**	oral	Zero	This medicine contains x mg of polysorbate* in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>. Polysorbates in this medicine may alter the effects of other medicines. Talk to your doctor or pharmacist if you are taking other medicines.	Although most available safety data is for PS 80 or 20, the package leaflet information should be used for all types of polysorbates unless omission is justified. May influence the pharmacokinetics of concomitant drugs (e.g. enhancement of gastrointestinal absorption). ** The type of polysorbate(s) (e.g. polysorbate 80 or 20) in the medicinal product should be mentioned here.*
	parenteral	Zero	This medicine contains x mg of polysorbate* in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>. Rarely, polysorbates can cause severe allergic reactions. If you have breathing difficulty or swelling or you feel faint, get medical help at once.	May influence the pharmacokinetics of concomitant drugs (e.g. brain uptake, inhibition of intramuscular absorption). Information on compatibility of the medical device type (if any) with the polysorbate in the product should be indicated. ** See above*
		10 mg/kg per dose	Polysorbates can have an effect on your circulation and heart (e.g. low blood	The risk of severe hypotension could be minimised by slowing down the infusion (by more than 5 minutes). Electrophysiological

			pressure, heart beat changes).	studies show cardiac depression in dogs and inhibition of hERG currents by polysorbates in vitro. The potential for torsades de pointes in humans is unknown. For risk minimisation, a SmPC warning on the risk of concomitant use of medications that prolong the QT/QTc interval should be considered.
		35 mg/kg/day	Ask your doctor or pharmacist for advice if you have a liver disease. This is because polysorbates can have an effect on the liver.	In neonates doses > 80 mg/kg/day of polysorbate caused severe (fatal) hepatotoxicity.
	Topical	Zero	Polysorbates can cause skin allergy (e.g. rash, itching).	

Scientific background

Introduction

Polysorbates are non-ionic surfactants widely used as excipients in oral, topical and injectable medicinal product formulations (Garidel et al, 2009 [36]). The focus in this report lies on polysorbate 20 and 80 as the most relevant polysorbate excipients, polysorbate 80 being by far the most used one (Arzneimittelinformationssystem, AMIS; March 2017).

Polysorbates are also widely used as an emulsifier, dispersant or solubiliser in many foods (E 433) and in a variety of cosmetic products.

Polysorbates are currently not listed in the Annex to the 'Guideline on Excipients in the label and package leaflet of medicinal products for human use' [1, 30]. However, Polysorbate was proposed to be on the list of excipients for which safety issues should be included in the guideline, notably on parenteral preparations for paediatric population (e.g. potential cardiotoxicity).

This report summarises updated toxicological and safety data on polysorbates 80 and 20 and provides a risk assessment concluding on thresholds for PIL warning.

1. Characteristics

1.1. Category (function)

Emulsifying agent; nonionic surfactant; solubilising agent; wetting, dispersing/suspending agent.

1.2. Physico-chemical Properties

Definition

Mixtures of partial esters of fatty acids, mainly oleic acid (PS 80) or lauric acid (PS 20), respectively, with sorbitol and its anhydrides ethoxylated with approximately 20 moles of ethylene oxide for each mole of sorbitol and sorbitol anhydrides.

Chemical Names and CAS Registry Numbers

Polyoxyethylene (20) sorbitan monooleate 9005-65-6

Polyoxyethylene (20) sorbitan monolaurate 9005-64-5

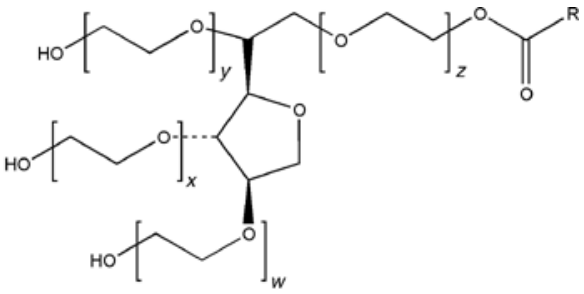
Empirical Formula and Molecular Weight

PS 80: $C_{64}H_{124}O_{26}$ Mr = 1310

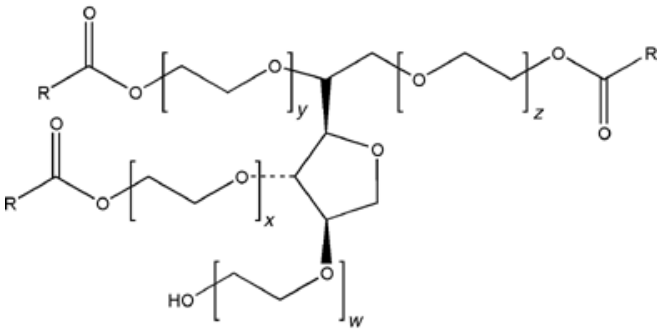
PS 20: $C_{58}H_{114}O_{26}$ Mr = 1228

147 **Structural Formula**

148



Polyoxyethylene sorbitan monoester



149 Polyoxyethylene sorbitan triester

150 w + x + y + z = 20 (Polysorbates 20, 40, 60, 65, 80, and 85)

151 w + x + y + z = 5 (Polysorbates 81)

152 w + x + y + z = 4 (Polysorbates 21 and 61)

153 R = fatty acid (Polysorbate 80: > 58% oleic acid; Polysorbate 20: 40-60% lauric acid)

154 **Typical Properties** (Ph .Eur. monograph 01/2017: 0426 and 0428; Handbook of pharmaceutical
155 excipients 2012 [42]; Wan and Lee; 1974 [105])

	Values (PS 80 / PS 20)
Acid value	≤2.0 for PS 80 and PS 20
Acidity/alkalinity	pH = 6.0–8.0 for a 5% w/v aqueous solution
Critical micelle concentration (CMC) at 25°C (µg/ml)	≈ 14 / 60
Flash point	149°C for PS 80
HLB value	15.0/16.7
Hydroxyl value	65–80/96-108
Moisture content	≤3.0 for PS 80

	Values (PS 80 / PS 20)
Saponification value	45–55/40-50
Solubility D = dispersible; I = insoluble; S = soluble; T = turbid; W = on warming.	 Ethanol = S Mineral oil = I Vegetable oil = I Water = S
relative density at 20°C	1.08 / 1.11
Surface tension at 20°C (mN/m) for 0.1% w/v solutions	42.5 for Polysorbate 80
Viscosity (dynamic) (mPa s)	425 / 400

Stability and Storage Conditions

Polysorbates are stable in the presence of electrolytes and weak acids and bases; gradual saponification occurs with strong acids and bases. Polysorbates are hygroscopic and should be examined for water content prior to use and dried if necessary. Upon storage, polysorbates are prone to oxidation and formation of peroxides (the oleic acid esters are sensitive to oxidation notably due to photosensitivity).

Polysorbates should be stored in a well-closed container, protected from light, in a cool, dry place.

Incompatibilities

Discoloration and/or precipitation occur with various substances, especially phenols, tannins, tars, and tarlike materials. The antimicrobial activity of paraben preservatives is reduced in the presence of polysorbates. Also, publications indicate auto-oxidation of polysorbate (80 or 20) in aqueous solution resulting in hydroperoxide formation, reactive aldehydes including formaldehyde and acetaldehyde, or side-chain cleavage, that could influence the stability of proteins (e.g. Maggio et al., 2012 [61]).

Polysorbates are widely used as a stabiliser for formulation of proteins avoiding their aggregation. Its compatibility with the active substance should therefore be demonstrated before any approval..

Also, polysorbate 80 is known to increase the rate of di-(2-ethylhexyl) phthalate extraction from polyvinyl chloride (PVC) materials (Takehisa et al., 2005 [94]). Therefore, the demonstration of the suitability of the primary packaging by compatibility studies including studies on extractables and leachables during pharmaceutical development is required where appropriate (Sharma, 2017 []).

1.3. Use in medicinal products

Polysorbates 20 and 80 are non-ionic surfactants which are widely used as excipients in oral, topical and injectable formulations (Garidel et al, 2009 [36]). For example, they are included in over 3000 medicinal products authorised in Germany (March 2017) and close to 2000 in The Netherlands. In medicinal products for oral use, Polysorbate 80 and 20 are used in coated and uncoated tablets, capsules, oral solutions and suspensions. The PS content is variable and ranges from 0.02 to 66 mg per dose in centrally authorised solid formulations. Polysorbates also serve as solubilising agents in

(many) injectable formulations of poorly soluble active substance (e.g. docetaxel, amiodarone). The highest PS exposure estimated was 55 mg/kg per dose (Taxotere(R), active substance docetaxel, 26 mg/mg PS 80 (ten Tieje et al. 2003a), 75 mg docetaxel/m2; 60 kg adult).

Polysorbates are also added in certain multivitamin solutions to dissolve liposoluble and hydrosoluble vitamins in the same medium. Furthermore, polysorbates are present in a large number of biological medicinal products such as enzymes (alteplase), immunoglobulins and monoclonal antibodies for both preventing surface adsorption and as stabilisers against protein aggregation. The concentrations range varies from 0.0003% to 0.3% (w/v) (Kerwin, 2008 [48]). Low amounts of Polysorbate 80 (20µg/ml) have been added to SCIG (Hizentra) to improve the visual appearance of the solution, as highly concentrated IgG solutions do not have a homogenous appearance (Maeder et al., 2011 [60]). PS exposure from therapeutic proteins is estimated to be much lower than from small molecules, i.e. up to 0.17 mg/kg per dose.

Furthermore, polysorbate 80 and 20 are used in vaccines, either as excipient during the production of the antigen preparation or as emulsifying agent in emulsion adjuvants For example, an oil-in-water (o/w) emulsion containing droplets of squalene surrounded by a monolayer of non-ionic surfactants polysorbate-80 and sorbitan trioleate (Span 85) (Shultze et al., 2008 [89]) or as an adjuvant system containing α-tocopherol and squalene in an oil-in-water emulsion. The oil phase is surrounded by non-ionic detergent polysorbate 80 (4.86 mg) (Langley et al., 2012 [56]).

Systemic exposure to polysorbates by most vaccines is low due to their low amount (up to 0.75 mg/kg) with usually higher levels (up to 4.85 mg/kg) for vaccines containing PS in the adjuvant system.

1.4. Regulatory status in food

In the EU, polysorbates 20, 40, 60, 65 and 80 were approved by the Directives for Food Additive (1995 [33]), and standards for their use were established (Annex to the Commission regulation (EU) No 1130/2011; Polysorbate 80 = E 433). In its scientific opinion, the EFSA Panel on Food Additives and Nutrient Sources Added to Food concluded that, based on the NOAEL of 2 500 mg/kg bw/day, identified from an oral carcinogenicity study with polysorbate 80 in rats, and applying an uncertainty factor of 100, a group ADI of 25 mg/kg bw/day for polysorbates 20, 80, 40, 60 and 65 (E 432, E 433, E 434, E 435 and E 436, respectively) could be established (EFSA 2015 [23]). It was further estimated that exposure of toddlers at the highest level was very close to the ADI (24.5 mg/kg bw/day).

1.5. Regulatory status in cosmetics

Polysorbates are used as hydrophilic, nonionic surfactants in a variety of cosmetic products. The Cosmetic ingredients expert panel reviewed the safety of polysorbates in 1984 [18]. The report states that these ingredients are used in numerous preparations without clinical reports of significant adverse effects. It was concluded that they are safe for use in cosmetics at present concentrations of use.

2. Pharmaco-toxicological data

Thorough reviews on the pharmacology/toxicology of polysorbates were conducted by the Joint FAO/WHO Expert Committee on Food additives (JECFA) in 1974 (JECFA - Joint FAO/WHO (Food and Agriculture Organization/World Health organisation): Toxicological Evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents, WHO Food Additive Series No 5. (1974)), the Cosmetic Ingredient Review (CIR) Expert panel (US) in 1984 (see above), the SCF) in 1985 and 1993 (see above) and the Japan Food Safety Commission in 2007

(Evaluation Report of Food Additives: Polysorbates (Polysorbates 20, 60, 65, and 80), Food Safety Commission (June 2007). These reviews were used as a basis for the assessment.

2.1. Pharmacodynamics and Safety Pharmacology

Polysorbates have been shown to activate or inhibit numerous biochemical reactions in vitro (enzymes, cellular respiration, DNA replication etc.) which may not be necessarily indicative of the in vivo effects of the polysorbates (CIR, 1984 [18]).

The pharmacodynamics effects observed on the cellular level in vitro or in vivo and the effective concentrations/doses are summarised in Table 2 (see end of section 2.1).

2.1.1. Biological membranes

Due to the surface active properties of the polysorbates and the physicochemical nature of cellular membrane bilayers, the polysorbates can affect the structure and function of biological membranes. Because of its dual hydrophobic/hydrophilic nature, polysorbate 80 in solution tends to orient itself so that the exposure of the hydrophobic portion of the molecule to the aqueous solution is minimised. Extensive studies have been made on the action of nonionic surfactants using test systems ranging from artificial lipid monolayers to natural multilayer epithelia.

Whether the effect the polysorbates have on membranes is solely a function of their hydrophile-lipophile balance or whether the specific structure of the polysorbate molecule may also determine its biological activity is unclear. For example it was concluded that the lysis of erythrocytes by the polysorbates was caused not by the destruction of the membrane but by some rearrangement of the membrane structure accompanying adsorption of the surfactant. Electrophysiological studies in artificial membranes indicated that polysorbates lower the conductance of the membrane by making it less permeable to charged molecules and decrease membrane stability by becoming incorporated into the membrane structure (CIR report 1984 [18]). Recent investigations indicated that polysorbate 80 may increase the susceptibility of cells to oxidative stress (Tatsuishi et al. [95]).

Jelinek (2001) thoroughly investigated the cytotoxicity of several tensides in vitro: The lowest observed half-maximum cytotoxic concentration (CC50) for polysorbate 80 was 0.048 mg/ml determined by a XTT-assay with U937 cells (human monocytic cell line) after 24h incubation. As with other tensides, apoptosis inducing properties were predominant at low concentrations whereas at higher concentration necrosis and cell lysis become more prominent.

In agreement to this, other investigators observed cytotoxic effects in vitro at similar concentrations of 0.1 mg/ml (e.g. Ménard et al. 2011 [68], HUVEC model, derived from LDH release) respectively.

The critical micelle concentration (CMC) for polysorbate 80 has been reported in the range of 14 µg/ml (12 µM) - 26 µg/ml (about 20 µM) (Wan and Lee, 1974 [105]; Kerwin, 2008 [48]; Ménard et al., 2011 [68]). Comparison of these findings with the cytotoxicity data discussed above shows that both apoptosis induction and cell lysis induction occurs at PS 80 concentrations above the CMC.

Many investigators have shown that PS 80 can interfere with the function of the transmembrane drug-export pump P-glycoprotein (P-gp, MDR1) either directly or through membrane perturbations, modulating multidrug resistence (MDR). Polysorbate 80 inhibits P-gp over a range from 0 to 1 mM, while it increased apical-to-basolateral permeability (AP-BL) and decreased basolateral-to-apical (BL-AP) permeability of the P-gp substrate rhodamine 123. These P-gp inhibition effects would appear to be related to these excipients' modulation of membrane fluidity, where PS 80 fluidises cell lipid bilayers. PS 80 also inhibits the peptide transporter, as measured by glycyl sarcosine permeability (Rege, 2002

[82]). Also polysorbate 20 has been reported (Yang et al., 2012 [110]) to increase significantly intracellular accumulation of doxorubicin in vitro via a possible mechanism of inhibiting MDR1 function and expression.

This MDR1-reversing ability was used to develop a bioassay for polysorbate 80 (Webster et al. 1997, see PK chapter). Complete reversal of MDR1 in vitro occurs at polysorbate 80 concentrations of 1-2 µl/ml, 50% inhibition occurs at levels of 0.2-0.3 µl/ml (corresponding to 0.22-0.33 mg/ml; Webster et al., 1997 [106]). Drori et al. (1995 [22]) demonstrated that Tween 80 alters membrane fluidity and increases membrane permeability and that these changes in the physical properties of biomembranes are important factors in achieving potentiation of anticancer-drug cytotoxicity.

A recent review (Zhang et al., 2016 [113]) suggests that polysorbates may interfere with the function of also other efflux proteins such as BCRP or MRP2 as well as metabolic enzymes in the CYP family (e.g. CYP3A4, CYP2C9). Specific polysorbates may differ in their activity profile with regards to which efflux transporters and/or metabolic enzymes are affected.

Polysorbates produce various, seemingly disparate effects in neuromuscular systems. Both stimulation of colonic motility but also clear spasmolytic activity of Polysorbate 80 was found in animal studies (CIR 1984 [18]).

2.1.2. Blood brain barrier

It has been known for a long time that polysorbate 80 increases the uptake of drugs into the brain (Azmin et al., 1985 [3]). Azmin et al. tried to investigate the mechanism by which polysorbate 80 enhances brain uptake of intravenous methotrexate (MTX) in mice. They could show that increased brain levels of MTX were observed after intravenous administration of MTX plus PS 80 compared to MTX alone, and the reverse was true for the MTX serum levels, indicating a direct effect of PS 80 on the BBB. This effect was observed with the lowest intravenous systemic dose of PS 80 investigated (3.2 mg/kg), lower doses of polysorbate 80 have not been tested. A possible enhancing effect by polysorbate 80 on the elimination of MTX from plasma was also discussed (Azmin et al. 1985 [3]). Calvo et al. (2001) [14] showed that a polysorbate 80 intravenous dose of 20 mg/kg in rats dramatically increased BBB permeability to sucrose. Polysorbate 80-coated nanoparticles can deliver drugs to the brain by a still debated mechanism (Kreuter, 2013 [54]). Gulyaev et al. (1999 [40]) had demonstrated that intravenous polysorbate 80-coated nanoparticles (coated by stirring in a 1% PS 80 solution) were able to deliver doxorubicin to the brain of rats. The highest levels were achieved between 2 and 4 h after drug administration. Administration of free doxorubicin in saline, or in 1% polysorbate 80 solution or loaded to non-coated nanoparticles could not enhance brain uptake. These data correlate with the notion that coated nanoparticles reach brain endothelial cells essentially intact. Adsorbed on the particle surface, PS 80 may be delivered more efficiently to the brain endothelial cell. This could explain why the addition of polysorbate 80 surfactant solution to free doxorubicin was totally inefficient.

Induction of endocytosis and/or transcytosis of the coated particles is favored as underlying uptake mechanism by polysorbate 80, but also membrane lipid solubilisation, opening of tight junctions or inactivation of the P-glycoprotein efflux pump could contribute to the effect (Kreuter, 2013 [54]). Polysorbate 80 stabilised nanoparticles adsorb preferentially apolipoproteins E or B that have been found responsible for the interaction with the BBB and the subsequent endocytosis/transcytosis (Göppert et al., 2005 [38]; Zensi et al., 2009 [111]). Recent investigations by Koffie et al. further supported that PBCA nanoparticles coated with polysorbate 80 do not induce nonspecific BBB disruption, but collaborate with plasma apolipoprotein E to facilitate BBB crossing (Koffie et al., 2011 [53]).

High accumulation of edelfosine in brain was reported by Estella-Hermoso de Mendoza et al. (2011 [29]). The authors suggested it was due to the inhibition of P-glycoprotein by Tween® 80, as verified using a P-glycoprotein drug interaction assay. In vitro studies revealed that edelfosine-loaded lipid nanoparticles induced an antiproliferative effect in C6 glioma cell line.

2.1.3. Cardiovascular effects

Hemodynamics

There have been several studies on the hemodynamic effects of the Polysorbates. The effects of the Polysorbates vary from species to species, with a general trend toward a depression of cardiac output. When a 5% aqueous solution of Polysorbate 80 was injected intravenously in doses of 1 ml/kg into cats, rabbits, and rhesus monkeys, there was a slight and transient fall in blood pressure; dogs exhibited a prolonged depressor response. This effect was never elicited by oral administration of the Polysorbates (CIR report 1984 [18]).

Masini et al. demonstrated that histamine release is the main cause of the cardiovascular effects of Polysorbate 80: Histamine releasing properties have been demonstrated in vitro on isolated mast cells and in vivo in the dog. Administration of a dose of 10 mg/kg to a dog over 5 min (equals to an infusion rate of 2 mg/kg/min) produced severe hypotension accompanied by an increase in plasma histamine. H1- and H2-receptor blockade significantly reduced the cardiovascular effects. The authors concluded that the hypotension induced by the commercial intravenous amiodarone in dogs and humans is not due to amiodarone but to its solvent PS 80 (Masini et al. 1985 [65]).

More recent findings in dogs by Cushing et al. (2009) also support that the hypotensive effects observed after amiodarone IV result from the cosolvents used in its formulation (i.e. polysorbate 80/benzylacohol): No significant hemodynamic changes were found in dogs using a novel intravenous cyclodextrin-based formulation of amiodarone compared to the response observed with the commercial US formulation (Abraxis®) as well as with a vehicle formulation containing PS 80 and benzyl alcohol only (Cushing et al. 2009). The Abraxis® dose of 2.14 mg/kg amiodarone (equals to a PS dose of 4.28 mg/kg) was given as a bolus push or as a 10 min infusion (rate: 0.43 mg PS/kg/min).

Cardiotoxicity

Non-clinical in-vitro and in-vivo electrophysiology

Polysorbate 80 (Tween 80) inhibits hERG currents with a half-maximally inhibitory concentration of 0.02% (IC20 0.001%; Himmel 2007 [44]). According to the author the inhibitory effect by polysorbate 20 is similar to (rather weaker than) PS 80. Part of the inhibitory effect is attributed to their interaction with lipid membranes, because hERG inhibition occurs close to critical micelle concentrations (Tween 20: ~ 0.007%).

Batey et al. (1997 [7]) found that 0.001% Polysorbate 80 in combination with 1% DMSO (vehicle for halofantrine) increased the effective refractory period in guinea-pig right ventricular strips and left papillary muscles; the authors concluded that “The ability of the vehicle to prolong the effective refractory period in the ventricular preparations may be due to blockade of an outward K+ current such as I_{Kr} (…) it would seem likely that the observed increases in effective refractory period in ventricular preparations could be due to DMSO”.

Torres-Arraut et al. (1984) [100] studied the electrophysiological effects of Polysorbate 80 in the cardiac conduction system of the dog and found that i.v. administration of 10 and 20 mg/kg (cumulative) Polysorbate 80 (equivalent to the amount of diluent in 5 and 10 mg/kg respectively of commercial intravenous amiodarone) induced prolongation of the sinus node recovery time, depressed

AV-nodal function and increased the atrial effective refractory period (ERP); most importantly, at 20 mg/kg polysorbate 80 increased ventricular ERP. The authors concluded that "The electrophysiologic effects with Polysorbate 80 are comparable to those of i.v. administration of pure amiodarone dissolved in distilled water to dogs", that "Serious complications such as A-V-block, hypotension and cardiovascular collapse have been associated with the use of the commercial intravenous form of amiodarone (…) these reactions could have been caused or potentiated by Polysorbate 80 is a possibility" and that "Polysorbate 80 is a potent depressant of the cardiac conduction system in the dog and its electrophysiologic effects are similar to those of amiodarone".

After i.v. administration (1 h infusion) of polysorbate 80 at doses of 3–4.5 g, end of infusion plasma concentrations of polysorbate 80 in humans were about 0.1 µl/ml (i.e. in the 0.01% range; Webster et al. 1997 [106]). At similar concentrations, polysorbate 80 was found to inhibit hERG currents (IC_{50} value of 0.02%; Himmel 2007 [44]). A retrospective analysis of literature data indicated that block of hERG currents is associated with life-threatening Torsades de Pointes (TdP) cardiac arrhythmias if it occurs at concentrations close to those achieved in clinical use, and a 30-fold margin between free therapeutic plasma concentrations and IC_{50} values for block of hERG currents appears to be a line of demarcation between the majority of drugs associated with Torsades de Pointes (TdP) arrhythmias and those which are not (Redfern et al., 2003 [81]).

The observation of Batey et al. (1997 [7]) that 0.001% Polysorbate 80 in combination with 1% DMSO increased the effective refractory period in guinea-pig right ventricular strips and left papillary muscles might be due to block of I_{Kr} (which is encoded by hERG in humans) by polysorbate 80, and not due to DMSO as suggested by the authors. Himmel (2007 [44]) found that DMSO at a concentration of 1% inhibits hERG currents only by 16%, and similar weak or absent effects of DMSO on hERG currents have been observed (Zünkler et al., unpublished observations). In contrast, polysorbate 80 at a concentration of 0.001% (those tested by Batey et al., 1997 [7]) has been found to inhibit hERG currents by 20% (Himmel, 2007 [44]).

The preservative chlorobutanol and the hERG channel pore-blocker terfenadine synergistically inhibit hERG currents (Friemel and Zünkler, 2010 [34]), and it might be speculated that similar synergistic effects on hERG channels might occur after administration of polysorbate 80 in combination with other hERG channel blockers. Given the proposed mechanism of action of polysorbate 80 on hERG channels (an interaction with lipid membranes (Himmel, 2007 [44])), the similar potency for polysorbate 80 to inhibit both hERG currents and MDR1 and the effects observed in dogs after i.v. administration of polysorbate 80 (depression of the AV-nodal function in addition to increasing the atrial and ventricular effective refractory periods), it is tempting to speculate that polysorbate 80 is a "multi-ion channel blocker" in the heart inducing cardiac electrophysiological effects not only via block of I_{Kr} (hERG channels).

2.1.4. Immune system

Complement activation

Weiszhár et al. (2012 [107]) provided experimental evidence that polyethoxylated surfactants, such as Polysorbate-80, activate the complement system in vitro, in normal human serum and plasma, generating the biologically active complement products, C3a, C5a and C5b-9.

PS 80 appeared to be more efficient reactogen than its structural homolog, PS 20. These results are consistent with the hypothesis that therapeutic side effects, such as acute hypersensitivity and anaphylactoid reactions, caused by intravenous medicines containing polyethoxylated detergents such as PS 80, can be attributed to complement activation-derived inflammatory mediators. Szebeni et al.

(2005 [92]) have tentatively named such reactions as “Complement activation-related pseudoallergy (CARPA)” as a new class of drug induced toxicity including amphiphilic lipids. Also Coors et al. (2005 [19]) identified Polysorbate 80 as the causative agent for an anaphylactoid reaction of non-immunologic origin in a patient (see 4.1.)

Immunosuppressant effect

Mice given 0.3 ml intraperitoneal injections of 25% polysorbate 80 in saline solution prior to immunisation with ovalbumin absorbed to aluminium hydroxide demonstrated no primary IgE response, indicating that Polysorbate 80 inhibited this response. Prior intraperitoneal injection of 0.3 ml of 25% Polysorbate 80 in saline also caused a total suppression of the primary IgG response and a partial suppression of the passive haemagglutination response to ovalbumin in mice. Jerne plaque assays showed significant suppression of the primary antibody response. Mice treated with Tween 80 showed no significant decrease in contact sensitivity. Thus, the suppression caused by Tween 80 affected only the primary humoral immune response (Bryant and Barnett, 1979 [13]; Barnett and Bryant, 1980 [6]; Barnett, 1981 [5]; text excerpt from CIR review, 1984 [18]).

Since very high doses of polysorbate 80 were used (intraperitoneal injection of 0.3 ml of 25% solution = 83.3 mg absolute dose of PS 80, corresponding to about 4167 mg/kg (!) assuming a mouse weighing 20 g), the clinical relevance of these findings for the use as an excipient is questionable.

2.1.5. Tumor promotion/Tumor growth inhibition

Numerous reports are available on tumor promotion and cocarcinogenesis by polysorbates after application to the skin. Polysorbate effects on the skin that have been linked to tumor enhancement were the induction of epidermal hyperplasia (possibly due to its effect on biological membranes), inhibition of DNA repair, or facilitation of direct contact of a carcinogen with mucosal cell surfaces (CIR report 1984 [18]).

Several studies have shown that the polysorbates at higher concentrations also have tumor growth inhibition activity. Tumour growth inhibition by Tween 80 was reported in mice: Intraperitoneal injection of polysorbate 80 into mice inoculated with carcinoma cells significantly reduced the formation and size of tumours and increased survival time of the animals (Witek et al. 1979 [109], Crispens et al. 1988 [20]). One author concluded that the cytotoxicity of polysorbate 80 for the tumour cells was related to the oleic acid component, since substitution of the polyoxyethylene sorbitan residue by diethanolamine did not eliminate the cytotoxic action (Witek et al. 1979 [109]).

Ng et al. (2004 [72]) demonstrated that the antiangiogenic property of taxanes can be significantly impaired by their formulation vehicles Cremophor EL and Tween 80, as well as serum binding proteins. The underlying mechanistic basis is unclear. Tween 80 itself caused significant inhibition of angiogenesis at ≥5 µl/ml (corresponding to 5.5 mg/ml).

Table 2. Effective concentrations of PS 80 in vitro

PD parameter	Effective Concentration observed (LOEL, IC50)*	Equivalent concentration in mg/ml*	reference
Micelle association	CMC 20 µM	0.026 mg/ml	Kerwin 2008
Cytotoxicity	Lowest CC50 48 µg/ml	0.048 mg/ml	Jelinek 2001

P-gp (MDR1) inhibition	IC50: 0.2-0.3 µl/ml	0.22-0.33 mg/ml	Webster et al. 1997
Cardiotoxicity (hERG-channel inhibition)	IC_{50} 0.02%	0.2 mg/ml	Himmel 2007
Haemolysis and cholestasis (isolated perfused rat liver)	1 µl/ml in perfusate	1.1 mg/ml	Ellis 1996
Histamine release (rat mast cells)	Lowest effect at 2.5 µl/ml 50% release at 25 µl/ml	2.7 mg/ml 27 mg/ml	Masini et al. 1985
Antiangiogenic effect (rat aortic rings)	Significant effect > 5 µl/ml	5.5 mg/ml	Ng et al. 2004

* relative density (20/20'C) of polysorbate 80 is about 1.1 g/ml (European Pharmacopoeia 8.1, 2014). Therefore, 1 µl of pure polysorbate solution equals 1.098 mg polysorbate.

Table 3. Effective doses of PS 80 in vivo

PD/Tox endpoint	Species	Admin route	NOAEL	Effective dose	reference
enhancement of brain uptake of other drugs	mice	i.v.		3.2 mg/kg (lowest dose tested; LOEL possibly lower)	Azmin et al. 1985
	rat	i.v.		20 mg/kg	Calvo et al. 2001
cardiac depression	dog	i.v.		10-20 mg/kg	Torres-Arraut et al. 1984
Hypotension (histamine release)	Dog	i.v.		10 mg/kg	Masini et al. 1985
Depression of primary immune response	mice	i.p.		>4000 mg/kg	Barnett et al. 1980
neurobehavioral toxicity (PS 80)	rats	p.o.	2,013 mg/kg/d		Ema et al. 2008
diarrhea	rats	p.o.	4,500 mg/kg/d		EFSA, 2015

2.2. Toxicology

2.2.1. Single Toxicology

Polysorbates have a very low acute toxicity in rats and mice via oral, IP and IV routes with an LD50 > 2 g/kg/bw (BIBRA, 1992 [8]; Farkas et al., 1991 [31])

2.2.2. Repeated Toxicology

Oral

Administration for three month with a dose of 10 mg/kg (0.2% wt/vol) polysorbate 80 in mice, rats, dogs, and monkeys was well tolerated (Thackaberry et al., 2010 [98]).

In a 13-week dietary administration study of polysorbate 80 (0.31%, 0.62%, 1.25%, 2.5% and 5%) in F344 rats, no macroscopic or histological changes were observed in any organs.

In a similar conducted 13-week study in B6C3F1 mice, no effects were observed (Food Safety Commission, 2007 [33]).

Degenerative lesions was reported in the heart, liver and kidney after a daily oral administration of 1.5 ml of 1%, 2% and 3% polysorbate 80 solution to rats for 3 months. However, no similar effects were seen in other studies (Food Safety Commission, 2007 [33]).

In mice given polysorbate 80 in the diet for 10 weeks reversible liver damage developed. Unfortunately, no dose was specified (BIBRA, 1992 [8]).

No effects were reported in oral toxicity studies in rhesus monkeys given polysorbate 80 0.1g/kg/day for 10 month, and in rabbits given polysorbate 80 3.6-55 g/kg/d for 65 days (BIBRA, 1992 [8]).

In repeated-dose toxicity studies diarrhea was observed as a major symptom. Based on the occurrence of diarrhea in rats fed polysorbates in subchronic studies, the lowest no observed adverse effect level (NOAEL) was calculated at 2% (polysorbate 60, equivalent to 1,460 mg/kg body weight/day; EFSA, 2015).

Parenteral

Increased heart and kidney weight was observed without cellular damage after intramuscular injection of 0.6 g/kg/day for 1842 days in rats (BIBRA, 1992 [8]).

Intravenous administration for 65 days in rabbits of 1.3–2 g/kg/day of polysorbate 80 (as a 20% aqueous solution) resulted in kidney and spleen injury (BIBRA, 1992 [8]).

Local Tolerance

P80 stabilised NLC toxicity has been investigated using in vitro Eytex test and Draize test and no or minimal irritancy potential was scored indicating minimal toxicity or irritation potential to the external ocular tissues (Gonzalez-Mira et al., 2010 [39]).

Moderate irritation has been observed after daily treatment for 1 month to rabbit skin with a solution of 5% of polysorbate 80. No effects were identified after daily application for 10 days (Anonymous 1984).

Genotoxicity

In genotoxicity studies (in vitro and in vivo), it was concluded that polysorbate 80 was not mutagenic (Food Safety Commission, 2007 [33]).

Carcinogenicity

Hyperplastic lesions in B6C3F1 were increased at 50,000 ppm in male and females after a 2 years administration via diet, without a carcinogenic potential. No tumor genesis was identified in G57BL mice after 10 weeks of treatment up to 100 mg/mouse/d (Food Safety Commission, 2007 [33]).

After dermal administration in mice (skin-painting of 80 mg of undiluted polysorbate 80 solution 6 times a week) for 52 weeks, only one mouse developed a benign skin tumor (Food Safety Commission, 2007 [33]).

A study in rats, after subcutaneous injection for 40 weeks of 2 ml of 6% polysorbate 80 solution (3 times a week) fibrosarcomas were identified at the injections site in 11 out 20 animals (Food Safety Commission, 2007 [33]).

No carcinogenic potential was demonstrated in hamster after intratracheal injection with 0,2 ml of 5% polysorbate 80 once a week for lifetime (Food Safety Commission, 2007 [33]).

Reproductive function toxicity

In reproductive studies performed with polysorbate 80 (oral route), there were no effects on fertility, reproductive function and in morphological development, survival and growth of fetuses. Polysorbate 80 was not teratogenic (Food Safety Commission, 2007 [33]).

In a previous study conducted by Enright et al. 2010 [27] in rats and rabbits after oral administration of 10 mg/kg of polysorbate 80 did not exhibit effects on fertility, or effects on early embryonic development in rats and no effects on embryo-fetal development in rabbits.

Neonatal/Juvenile Toxicity

Gajdova et al. (1993 [35]) investigated the influence of polysorbate 80 on the development of reproductive organs in neonatal female rats. Polysorbate 80 (1, 5 and 10% solution) was injected intraperitoneal for four days (days 4, 5, 6 and 7 after birth) and monitored up to 20 weeks. Statistical significant changes in vaginal opening time were observed for the medium and high dose group. In untreated animals the average length of the oestrous cycle was 4.3 compared to the range from 9.3 to 14 days in the treated animals. Further a decrease in relative weight of the ovaries in all groups treated with polysorbate 80 was found compared to the control group. The authors concluded that the identified changes were similar to that what have been seen after the administration of diethylstilbestrol, which was used as a positive control. Williams et al. (1997 [108]) could not confirm, after oral administration (up to 5 g/kg/d) for 3 days to immature rats (21 days after birth), the estrogenic effects of polysorbate 80 as it was observed in previous study by Gajdova et al. [35], after intraperitoneal injection in neonatal female rats.

Investigation in young piglets (2 days after birth) that were treated with 2 to 4 ml/kg/day up to 13 days with a mixture of polysorbate 80 and polysorbate 20 after intravenous administration was found as not toxic. But the authors noted a recurring of subcutaneous edema in the neck region and several cases of necrotising enterocolitis at autopsy (Hale et al., 1995 [41]).

There are several reports on neurobehavioral toxicity of polysorbate 80 available, conducted in rats, mice and cats (see Ema et al., 2008 [25]). To further evaluate the developmental neurotoxicity, including locomotor activity, of polysorbate 80 a study in rats was conducted by Ema and Coworkers (Ema et al., 2008 [25]). Polysorbate 80 was given in their drinking water at concentration of 0.018, 0.13, 1 and 7.5% during pregnancy and lactation (day 0 of pregnancy until day 21 after delivery). For the locomotor activity no changes in male and female animals were observed during the treatment period. However a decrease in successful conditioned avoidance responses was seen in the high dose group (7.5%), but no neurological changes were detected, including histophathological examination. The NOAEL considered being 1% (1,864 ml/kg/day) which is equivalent to 2,013 mg/kg/day. However, not enough information was provided if pups were directly exposed with Polysorbate 80 via maternal milk. The authors postulated that exposure took place partly via milk.

As pups gradually starts to consume food and water from around postnatal 14, it can be assumed that the pups were exposed with Polysorbate 80 onwards from this age via the drinking water (OECD 2008 [74]).

The potential neurodevelopmental and maternal toxicity was studied in animal model e.g. by Brubaker et al. (1982 [12]. It occurred at the 7.5 vol% treated group. In a ≤1% treated group, there was no effect on the mother rats and their subsequent generations (F1).

Farkas et al. [31] conducted a study to assess the toxic effect of a 9:1 polysorbate 80: 20 mixture (2.5–4 mg/kg) in newborn rats, 2 days old, after a single intraperitoneal injection (Farkas et al., 1991 [31]). The LD50/90 for neonatal rats was 3.5 g/kg. The main toxic effects observed in newborn rats were chylous ascites (milky fluid in the abdominal cavity), peritoneal fibrosis and severe tail inflammation. The authors concluded that the LD50/90 for neonatal rats is similar to that of adults.

Comparative toxicity study of i.v. administered alpha-tocopherol and alpha-tocopherol acetate and polysorbate vehicle containing formula (similar to that used in commercial preparations) was carried out on newborn rabbits by Rivera and co-workers. No toxicity could be attributed to the vitamin E or polysorbate treatment, but the polysorbate containing formula treated pups had microscopic evidence of mild bile stasis and elevated serum bilirubin levels, and lipidosis in the adrenal gland was primarily observed also in this group (Rivera et al., 1990 [84]).

2.3. Toxicokinetics

2.3.1. Oral administration

Studies have shown after oral administration in rats that the ester bond sites of polysorbate are hydrolyzed by pancreatic lipase and the free fatty acids then absorbed from the digestive tracts and oxidised. Excretion is mainly via exhaled breath as carbon dioxide. The kinetic is almost similar as observed for the metabolism of ordinary fatty acids. The efficiency with which rats hydrolyzed and absorbed the labeled fatty acid portions of polysorbate 80 when fed at a dietary level of 10% was 100%. The polyoxyethylene sorbitan moiety left after hydrolysis of the ester is poorly absorbed from the rat's gastrointestinal tract. In one study with a radioactive carbon label in the polyoxyethylene portion of polysorbate 20, 90% was excreted in the feces and 8% in the urine. No radioactivity was found in the liver, carcass, or expired CO2. When the sorbitol moiety of polysorbate 80 was labeled, 91% of the radioactivity was recovered in the feces, 2.1% in the urine, 1.6% in the carcass, and none in expired CO2, liver, kidney, spleen, adrenals, brain, gonads, or fat. Since the radioactivity of both the sorbitol and polyoxyethylene labeled polysorbates is found largely in the feces and not in respired air, it is evident that there is no splitting of the ether bond between the oxyethylene group and the sorbitan moiety (Treon et al., 1967 [101]; Cosmetic ingredients expert panel review, 1984 [18]; Food Safety Commission, 2007 [33]).

These observations suggest that oral bioavailability of intact polysorbate 80 is extremely low. This is in agreement with data in mice where only about 3.2% of total oral administered polysorbate 80 was found to be excreted unchanged in the urine (Azmin et al., 1985 [3]). Also the hydrolised polyoxyethylene moiety appears to be poorly absorbed (< 10%) and is mainly excreted as such in the feces (Treon et al., 1967 [101]). In conclusion, after oral exposure almost solely the released fatty acid becomes systemically available. This substantiates the very low oral toxicity of polysorbate 80.

2.3.2. Intravenous administration

After intravenous injection into rats, the ester bond is also hydrolyzed by blood lipases. When polysorbate 20 was injected into rats, the labeled lauric acid moiety was metabolised and appeared mostly as expired CO2 (68%; carcass, 22%; urine, 5%; feces and gastrointestinal contents, 2.5%; and liver 0.7%). The polyoxyethylene moiety was not catabolised, since no radioactivity was recovered as CO2 when this portion of the molecule was labeled. Most of the labeled polyoxyethylene (83%) appeared in the urine but some was present in the feces (11%) indicating biliary excretion (Treon et al., 1967 [101]).

Data in mice have shown that polysorbate 80 is also rapidly degraded after intravenous administration by esterases in plasma (van Tellingen et al 1999). The animals received an i.v. bolus dose of 3.3 µl of polysorbate 80:ethanol:saline (1:1:2, v/v/v) per g of body weight, corresponding to the amount of vehicle administered to animals receiving 33 mg/kg of docetaxel (0.83 µl/g, corresponding to about 0.9 g/kg). Within 15 min after bolus injection, the concentration of intact polysorbate 80 measured by HPLC rapidly declined to levels < 0.05% (v/v) of the plasma volume. Parallel results obtained by studying the in vitro kinetics of POLYSORBATE 80 breakdown strongly suggest that esterases in plasma, catalyzing the cleavage of the oleic acid side chain from the POLYSORBATE 80 molecule, are responsible for this rapid decay.

However, discrepancy is noted between the duration of 4 h for 50% breakdown by human plasma or pure esterase determined in vitro and the much more rapid decline observed in vivo.

3. Pharmacokinetics (in humans)

3.1. ADME (absorption, distribution, metabolism, elimination)

3.1.1. Oral administration

Clinical tests have shown that essentially the same pattern of metabolism is followed in humans as in the rat. The ethoxyl values of the urine and stools of four subjects fed 4.5 g of polysorbate 80 per day were determined to ascertain the amount of the polyoxyethylene portion excreted. The results showed that the polyoxyethylene fraction was excreted quantitatively; approximately 95% was excreted in the feces and 5% in the urine. Since there were no polyoxyethylenated fatty acids detected in the urine, it was concluded that the polyoxyethylene moiety in the urine represented polyoxyethylene sorbitan and not the parent ester. The Polysorbate 80 was most likely hydrolysed by pancreatic lipase, with the liberated oleic acid following the normal metabolic pathways of unsaturated fatty acids. The source of the polyoxyethylene in the urine was that portion absorbed from the upper intestinal tract following hydrolysis of the ester bonds, since there was no carryover of the polyoxyethylene sorbitan in the urine during the postmedication control periods, there was no storage of this moiety in the body.
The possibility of oxalic acid poisoning from the polyoxyethylene component would seem negligible in light of its quantitative excretion. Urinary studies for oxalate content in patients taking oral Polysorbate 80 indicated no increase in oxaluria (copied from: Cosmetic ingredients expert panel review 1984 [36]).

3.1.2. Intravenous administration

Webster et al. (1997 [106]) measured polysorbate 80 plasma concentrations in patients following i.v. administration of etoposide or docetaxel formulated with polysorbate 80 using a bioassay with MDR1-expressing cells (i.e. polysorbate 80 in plasma was determined on the basis of its ability to reverse

MDR1). Patients received docetaxel containing 3.12 – 4.45 ml polysorbate 80 (corresponding to 3.4-4.9 g), and the median end-infusion (1 h) polysorbate 80 concentration was 0.1 µl/ml (corr. to 0.1 mg/ml) (range 0.07 – 0.41 µl/ml; only 1 patient had a level of > 0.2 µl/ml). Patients received i.v. etoposide containing 0.67 – 0.93 ml polysorbate 80, and in the end-infusion plasma sample polysorbate 80 was not detectable (< 0.06 µl/ml). No time points other than "end of infusion" were investigated.

Sparreboom et al. have developed and published a method for determination of polysorbate 80 in plasma samples by liquid chromatography-tandem mass spectrometry (Sparreboom A et al., 2002 [91]). By using this method, human PK data for PS 80 (Tween 80) after single dose IV infusion of different doses of docetaxel (Taxotere®) have been obtained from 39 cancer patients (ten Tieje et al. 2003b [97]). Noncompartmental analyses yielded mean values of 7.7. L/h for total plasma CL, 3-8 L for Vss and 0.6h for the terminal half-life. Plasma exposure (C_{max} and AUC) increased linearly with dose. After the lowest single dose of PS 80 (drug dose: 25 mg/m2, Tween80 content 26 mg/mg drug-> PS 80 dose 650 mg/m2) mean plasma Cmax after 1 h infusion was 0.139 mg/ml (n=3), and after the highest individual dose (drug dose 75 mg/m2 -> polysorbate 80 dose 1950 mg/m2) mean plasma Cmax (n=19) was 0.457 mg/ml.

These intravenous human data provided evidence that polysorbate 80 has a high plasma clearance and very short half-life (< 1h) and that the relative systemic exposure is much lower than with similar excipients as Cremophor EL.

Apart from the data described above by the Sparreboom group no further human PK data of any polysorbate excipient after intravenous administration have been published to date.

3.1.3. Intramuscular administration

No data on the systemic bioavailability of polysorbate 80 after intramuscular injection (e.g. with vaccines) are available.

3.2. PK in children

In general, for many PK variables (also plasma esterase activity) there are clear differences between neonates and older infants and children (Morselli, 1976 [69]). Ester hydrolysis is low in newborns and it appears to be significantly related to the developmental stage. Reduced activity of acetylcholinesterases and arylesterases in premature and full-term newborns are reported. The progressive increase in esterase activity with age was paralleled by the increment in plasma proteins; both parameters achieving adult values at 1 year of age (Morselli, 1976 [69]). Whether this holds true also for plasma esterase/lipase hydrolising PS 80 is not clear.

As a conservative conclusion in analogy to other enzyme activities, in children < 1 year of age intravenous polysorbate 80 might be expected to be metabolised more slowly bearing a higher risk for adverse effects.

Pesce and McKean (1989 [79]) reported cases of death of several neonates after parenteral administration of a vitamin-E preparation (E-ferol), which contained 9% of polysorbate 80 and 1% polysorbate 20. Analysis of peritoneal fluid from a baby given E-ferol, a vitamin E supplement, revealed levels as high as 100 µg/ml polysorbate (McKean and Pesce, 1985 [67]). According to the authors the reason for this appears to be in the inability of the neonate to metabolise the compound, however, they do not provide any data supporting this assumption. Of note, the authors state that studies in rat pups are invalid to study neonatal toxicity, since most experiments are done on rat pups which are much more mature compared to human preterm neonates.

Interestingly, based on the published case report of 36 low-birth weight, premature neonates who experienced toxicity and death following receipt of high doses of an intravenous vitamin E product (E-Ferol) containing polysorbate 80 for a period ranging from 6 to 45 days, the toxicity (e.g. the development of hepatic lesions) and death could occur several months after the cessation of E-Ferol (Martone et al., 1986 [62]). Therefore, monitoring plasma concentrations of polysorbate 80 (e.g. during a study treatment period) may not necessarily help identify early signs of toxicity.

3.3. Interactions

Polysorbate 80 is reported to influence the pharmacokinetics of other drugs. Surface active agents are thought to produce micellar solutions in the intestinal lumen in much the same way as bile salts, thus enhancing the uptake of fatty acids. When fed to rats for 1 week at 0.1% and 1% of the diet, Polysorbate 80 augmented the absorption of fats present at 10 to 33% of the diet (CIR report 1984 [18]).

It has been known for a long time that polysorbate 80 also increases gastrointestinal absorption of other drugs as well as the uptake of drugs into the brain (Azmin et al., 1985 [3]). This ability is utilised in the coating of nanoparticles with PS 80 for drug delivery to the brain (Kreuter, 2013 [54]).

At 0.01% in human serum, PS 80 decreased the binding of atropine sulfate to serum albumin (CIR report 1984 [18]).

The ability of polysorbate 80 to form micelles leads to drug entrapment, significantly altering the disposition of the formulated drugs (ten Tije et al., 2003a [96]; Loos et al., 2003 [59]). In patients who received the same amount of polysorbate 80 that was present in 100 mg/m2 of intravenous etoposide, both the volume and the clearance of doxorubicin were increased (Cummings et al., 1986 [21]).

The pharmacokinetic study by Wang et al (Wang et al. 2012) showed that the polysorbate 80 coated poly (-caprolactone)–poly (ethylene glycol)–poly (-caprolactone) micelles altered the biodistribution pattern and increased paclitaxel concentration in the brain significantly compared to the uncoated micelles and the free drug after intravenous injection in rats.

However, PK investigations of Docetaxel and PS 80 in mice by van Tellingen et al. (1999 [103]) indicated that the vehicle was not able to interfere in the disposition of docetaxel due to the rapid degradation of polysorbate 80 by esterases in plasma (in contrast to Cremophor EL, which was found to be causative for the observation of nonlinear kinetics of Paclitaxel). Because of the fact that patients receive docetaxel by a 1-h i.v. infusion instead of a bolus injection (mice), the plasma levels of PS 80 remain much lower. Therefore, the authors conclude that in patients interactions by polysorbate 80 are even less likely.

On the other hand, Baker et al. (2005) observed an association between polysorbate AUC and unbound clearance of the drug docetaxel in patients with normal liver function.

There are two studies on the mechanism of the inhibitory effect of Polysorbate 80 on the intramuscular absorption of drugs. The inhibition of absorption could not be attributed to a direct or indirect effect on the capillary wall. It was concluded that the effect was mainly due to its influence on the extracellular space and the permeability of connective tissue (Kobayashi et al., 1974 [51]; Kobayashi, et al. 1977 [52]).

Polysorbate 20 and 80 inhibit P-glycoprotein (P-gp/MDR1) thereby influencing intracellular accumulation of drugs and modulating multi-drug resistance. Furthermore, polysorbates may interfere

with the function of also other efflux proteins such as BCRP or MRP2 as well as metabolic enzymes in the CYP family (see chapter 2.1.1., review by Zhang et al., 2016 [113])

Effect of polysorbate 80 on metabolic activity of CYP3A4 and CYP2C9 in human liver microsomes has been revealed by Christiansen et al (Christiansen et al., 2011 [16]). Polysorbate 80 inhibited of CYP3A4-mediated 6β-hydroxylation of testosterone with an IC50 value of 0.40 mM. IC50 concerning the CYP2C9-mediated 4-hydroxilation of diclofenac has been found 0.04 mM for polysorbate 80. Both IC50 values are below of the CMC of polysorbate 80. Effect of polysorbate 80 on the expression of CYP3A4 mRNA and CYP3A4 protein has been studied by Tompkins et al in HepG2 and Fa2N4 human liver cell lines, human primary hepatocytes and intestinal LSI74T cell model (Tompkins et al., 2010 [99]). Polysorbate 80 tended to decrease CYP3A4 mRNA and protein expression in the above mentioned model systems.

In a study in 8 patients with recurrent stage pTa or pT1 transitional cell carcinoma of the bladder the rate of absorption of thioTEPA was not influenced by Tween 80, but it did cause statistically significant increases in mean peak plasma levels (from 101 to 154 ng/ml) and mean AUC values (from 0.376 to 0.496 micrograms h per ml) and a decrease in the mean half-life (from 1.83 to 1.25 h). The authors concluded that to obtain plasma levels similar to those achieved after instillation with thioTEPA alone, the dose should be reduced with Tween 80 (Masters et al., 1990 [66]).

4. Clinical safety data

4.1. Safety in adults

Hypersensitivity, pseudoallergy

Early in the clinical development of docetaxel, it became clear that docetaxel administration is associated with the occurrence of unpredictable (acute) hypersensitivity reactions, neutropenia, neurotoxicity, musculoskeletal toxicity and cumulative fluid retention. These side-effects have been attributed, in part, to the presence of polysorbate 80 (Engels et al., 2007 [26]; Zhang et al., 2014 [112]).

The potency of polysorbate 80 as a type IV allergen is well-known. Tuberculin type hypersensitivity to PS 80 has been reported after water base formulated retinol injection to psoriatic patients and contact sensitivity to PS 80 by patch testing patients with eczema. Also a high sensitisation rate to emulsifiers like PS in patients with chronic leg ulcers was found (Pasche-Koo et al. 1994). Similar reactions were observed after i.m. injection of Vit. K (Aquamnophyton) with polysorbate 80 and not with preparation free of emulsifiers (Shelley et al., 1995 [88]). Few reports on polysorbate-induced contact urticaria exist (see Coors et al., 2005 [19]).

Furthermore, Coors et al. (2005 [19]) identified Polysorbate 80 as the causative agent for an immediate-type allergic shock reaction occurring in a patient after infusion of a multivitamin preparation containing polysorbate 80 (Multibionta N). No.Polysorbate-specific IgE antibodies were identified, confirming the non-immunologic nature of the anaphylactoid reaction.

Owing to polysorbate 80 in its formulation, even prophylactic pre-medications were administered to prevent hypersensitivity reactions (Hennenfent and Govindan , 2005 [43]). A positive prick test performed with polysorbate 80 has indicated the role of this substance in the development of urticaria in a 28 year old adult after injection of Humira® and Stelara® (Perez-Perez et al., 2011 [78]).

The mechanism of pseudoallergy caused by the polyoxyethylene nonionic surfactant was recently investigated by Li et al. (2014 [58]). Based on in vitro cell analysis, it was assumed that the initial

728 contact of polyoxyethylene nonionic surfactant with mast cells provoked pseudoallergy via polyamine
729 receptor-mediated endocytosis.

730

Hepatotoxicity / cardiovascular effects

Amiodarone

Rhodes et al. (1993 [83], case report of a 72y old adult) were the first to suggest polysorbate 80 as the hepatotoxic component in the IV formulation of amiodarone. Further case reports were published (Fonseca et al., 2015 [32]; Paudel et al., 2016 [77]; Ratz Bravo et al., 2005 [80]; Chen et al., 2016 [15]; Giannattasio et al., 2002 [37]).

In one case the patient was loaded with amiodarone 150 mg IV followed by amiodarone drip (1 mg/min for first 6 hours and then 0.5 mg/min for next 18 hours), a total dose of 1050 mg amiodarone was calculated (Paudel et al., 2016 [77]). This amiodarone dose corresponds to a cumulative dose of 2100 mg PS 80, i.e. 35 mg/kg in a 60 kg adult (content of 2 mg PS 80 per mg amiodarone assumed as in Cordarone®). A similar dose level is identified in a second case report (Fonseca et al., 2015 [32]): The patient was started on intravenous amiodarone with a bolus dose (injection over 3 min) of 300 mg followed by a continuous infusion of 900 mg over 24 h (1200 mg total dose amiodarone corresponding to 2400 mg PS 80, i.e. 40 mg/kg in a 60 kg adult). 18 h after starting amiodarone he showed an abrupt elevation of aminotransferases. As already shown in other cases, introduction of oral amiodarone in this patient did not result in any additional liver injury. Based on this observation, Rhodes et al. (1993 [83]) had proposed that polysorbate 80, the solvent of intravenous formulation of amiodarone, could be involved in this adverse effect since it is present in the intravenous but not in the oral form of amiodarone.

Munoz et al. (1988 [70]) investigated in 20 patients undergoing coronary arteriography the hemodynamic effects of an experimental preparation of i.v. amiodarone 5 mg/kg without Tween 80 (N) (10 patients) with those of the commercial form with Tween 80 (A) (10 patients). Both A and N caused similar bradycardia, increase in ventricular filling pressure, vascular resistance and decrease in cardiac and contractility indexes. Amiodarone blood levels were similar after A or N. The data document a significant initial short duration vasoplegia, mainly related to Tween 80, after A, when amiodarone itself after producing a similar very slight effect causes bradycardia, and a moderate and progressive negative inotropic effect. Both preparations were injected as 3 min bolus, thus rate of PS 80-injection was 3.33 mg/kg/min. It was concluded that while the experimental form would be of interest, the risk of severe hypotension after i.v. Cordarone can be largely avoided by using a slower rate of infusion, especially in patients with hypovolemic status (Munoz et al., 1988 [70]). The observations are supported by earlier studies from Sicard et al. (1977 [90]) who found vasodilatation with associated tachycardia when injecting five patients with a quantity of pure Tween 80 equivalent to the amiodarone formulation.

Docetaxel

Some adverse effects occurring in the majority of cancer patients receiving Taxotere®, such as severe hypersensitivity reactions and fluid retention, are considered attributable to the excipients polysorbate 80 and ethanol. For that reason, many polysorbate-free formulations are currently under development (e.g. Li et al. 2014 [58]).

Tagawa et al. (2017 [93]) compared the adverse event profiles following injection of original or generic docetaxel in breast cancer patients. Significant product-related differences were observed in the following non-hematological adverse events: injection site reaction ($P = 0.0012$), hand-foot syndrome (≥grade 3) ($P = 0.0003$), and oral mucositis (≥grade 3) ($P = 0.0080$). Multivariate logistic regression analyses identified significant negative associations with the amounts of polysorbate 80 and ethyl alcohol present (Tagawa et al., 2017 [93]).

Taxotere® leads to the highest PS exposure of all parenteral products (55 mg/kg; see Table 1). Impairment of liver function is among the common side effects. However, since docetaxel itself is potentially hepatotoxic, clinical cases of severe hepatotoxicity after docetaxel would not be attributed solely to polysorbate 80.

Vaccine-induced narcolepsy

In 2012, observational studies in Finland Sweden reported an association between the occurrence of narcolepsy (chronic sleep disorder with excessive daytime sleepiness) and vaccination with a European A(H1N1) pandemic vaccine (Pandemrix®) during the H1N1 influenza pandemic 2009. In the following, several large epidemiological studies in other European countries confirmed an increased risk of narcolepsy in children, adolescents and adults after vaccination with AS03-adjuvanted pandemic vaccine Pandemrix. In search of possibly causative ingredients, also a contribution of the PS 80 containing emulsion adjuvant (AS03) has been addressed. Vaarala et al. (2014 [102]) found detergent-induced antigenic changes of viral nucleoprotein (NP), that are recognised by antibodies from children with narcolepsy, these results moved the focus from adjuvant(s) onto the H1N1 viral proteins. Since in contrast to Pandemrix® after vaccination with Arepanrix® (also adjuvanted with polysorbate 80 containing AS03) or Focetria® (aduvanted with polysorbate 80 containing MF59) only few cases of narcolepsy were reported, the difference between these vaccines came into focus (Jacob et al., 2015 [45]). Recent data indicate that Pandemrix-induced narcolepsy might be caused by an immune response against NP which is present in much higher amounts in Pandemrix than in Focetria (Ahmed and Steinman 2016). Besides, Saariaho et al. (2015 [85]) found that patients with Pandemrix-associated narcolepsy had more frequently (14.6%) anti-GM3 antibodies than vaccinated healthy controls (3.5%) ($P = 0.047$). The data suggest that autoimmunity against GM3 is a feature of Pandemrix-associated NC and that autoantibodies against gangliosides were induced by Pandemrix vaccination. Altogether, there is currently no evidence for a causative contribution of polysorbate to Pandemrix®- induced narcolepsy.

It has to be noted that the content of polysorbate is markedly higher in vaccines where it is used as emulsifier in its oil-in-water adjuvant (1.175–4.85 mg/kg) than in many other vaccines where it is used as protein stabiliser (<< 1 mg/kg).

4.2. Safety in children

Parenteral Vitamin solutions (E-Ferol tragedy)

The hepatotoxic potential of polysorbate gained notoriety after the **E-ferol tragedy** in the 1980s. E-ferol was an IV formulation of **vitamin E** marketed in December 1983 as antioxidant therapy for premature infants. The formulation contained a mixture of polysorbate 80 (9%) and polysorbate 20 (1%) as solubilising agents. After only 4 months of use, 38 infant deaths were reported in 11 states. While hepatic histology results from infants receiving E-ferol suggested a more cytotoxic than steatotic process, few investigations supported vitamin E content as the responsible culprit, thus leaving the mixture of polysorbate as suspect. Neonates whose deaths were attributed to E-Ferol administration received 100 to 548 mg/kg polysorbates per day (25 to 137 vitamin E U/kg/day), the duration of therapy was from 6 to 45 days, and the cumulative dose of polysorbates ranged from 1508 to 12000 mg/kg (377 to 3000 vitamin E U/kg) (Bove et al. 1985). A clear dose-response relationship was found: the attack rate (no. cases/no exposed %) increased at average daily doses > 20 U/kg/d E Ferol. This corresponds to an increased risk at a PS dose of > 80 mg/kg/d (consisting of 72 mg PS 80 plus 8 mg PS 20) (Martone et al., 1986 [62]).

Polysorbates are still used as solubilising agents in marketed parenteral vitamin products in the US (M.V.I. Pediatric®: Multi-Vitamin for Infusion) that are administered to neonates. For example, the recommended daily doses of M.V.I. pediatrics® for infants < 1 kg are 30% (1.5 mL) of a single full dose (5 ml), and for infants weighing 1 to 3 kg 65% (3.25 mL) of a single full dose (5 mL) (M.V.I. pediatrics® label; 5 ml of reconstituted product provides 50 mg PS 80 and 0.8 mg PS 20 per 5 ml dose, in sum 50.8 mg polysorbates per 5 ml dose). This would yield an amount of 15.24 mg PS per dose for infants < 1 kg, which is equal to 30.5 mg/kg/d for an infant weighing 500 g. The polysorbate 80 dose for infants weighing 1-3 kg would be in the same range (11-33 mg/kg/d).

Amiodarone

Kicker et al. (2012) report of hepatotoxicity in a 34-week-old female infant with Down syndrome (2.6 kg) after amiodarone infusion. The reported patient received 270 mg of polysorbate 80 (103 mg/kg) in addition to 135 mg of IV amiodarone. The short-terminal half-life was discussed to explain the rapid resolution in hepatic injury after discontinuation of parenteral amiodarone.

Masi et al. (2009) published a case report of a 4-day-old newborn with cardiogenic shock after receiving by mistake a high “oral” loading dose (47 mg/kg) of amiodarone i.v. Considering that the injectable product has a ratio of 2 mg polysorbate 80 for every 1 mg of amiodarone, the newborn received ca. **100 mg/kg PS 80** i.v. with the amiodarone loading dose over a 30 min period (PS infusion rate ca. 3.3 mg/kg/min): Measured plasma concentrations of amiodarone never reached toxic levels. Unfortunately, PS levels were not measured. Of note, amiodarone IV solutions also contain the excipient Benzyl alcohol (Masi et al. 2009).

Anidulafungin

Cohen-Wolkowiez et al. (2011) investigated the pharmacokinetics and safety of anidulafungin in infants and neonates. Anidulafungin was administered intravenously to 15 infants and neonates over 3 to 5 days as a loading dose of 3 mg/kg infused over 60 min on day 1 and daily maintenance dosages of 1.5 mg/kg infused over 60 min. Two anidulafungin presentations were used in the study. Infants received an intravenous alcohol (20%) based presentation whereas neonates received an alcohol-free, water for injection presentation. Only one of the two anidulafungin presentations is still approved commercially (Ecalta®). No drug related serious events were observed. Eight out of 15 subjects (53%) experienced at least one adverse event; most of these events were mild or moderate in severity (Table 3, see end of section 2.1). All but 2 adverse events were considered by the investigator to be unrelated to anidulafungin. The most commonly reported non-serious adverse event was worsening hyperbilirubinemia.

From the content of polysorbate 80 in one of the products used (Eraxis® label) the exposure of PS 80 in this study was calculated to be 7.7 mg/kg/day on day 1 and 3.8 mg/kg/day over 3-5 days. As administrations were over 60 minutes these doses are equivalent to a rate of 0.064 – 0.13 mg/kg/min.

Parenteral nutrition

Total parenteral nutrition (TPN) is widely used. Although mechanical, septic, and metabolic complications are well known, hypersensitivity skin reactions are rare. The report of Levy and Dupuis (1990) describes a 16-year-old boy with Burkitt's lymphoma who developed an urticarial skin rash when treated with TPN and vitamins. The adverse skin reaction was probably caused by the inactive component of excipient, polysorbate.

5. Safety information relevant for the package leaflet

With respect to derivation for thresholds triggering a warning statement in the PI, a risk assessment for PS 80 (20) is warranted. For that purpose the potential hazard and the corresponding doses/concentrations with regard to different administration routes are summarised.

Topical exposure

Delayed hypersensitivity reactions including contact dermatitis and contact urticarial have been reported after administration of creams containing polysorbates. Therefore, a warning on allergic reactions at threshold zero is proposed.

Oral exposure

In contrast to the parenteral route the oral application appears to be much less toxic. This is probably attributed to the very low oral bioavailability of the intact polysorbate: Only small amounts of polyoxyethylene sorbitans are absorbed intact. Enzymatic cleavage in the gut leads to the fact that after oral exposure almost solely the released fatty acid becomes systemically available (see PK chapter).

In its recent re-evaluation EFSA sums up that similar toxicokinetics would be expected for all polysorbates based on their similarities in structure and metabolic fate. The acute toxicity is very low. There is no concern regarding genotoxicity, carcinogenicity or developmental toxicity. From a limited number of studies, there is no indication of reproductive toxicity (EFSA, 2015). In the re-evaluation by EFSA in 2015 the acceptable daily intake (group ADI) for polysorbates as food additives (polysorbates 20, 80, 40, 60 and 65; E 432, E 433, E 434, E 435 and E 436, respectively) was set to 25 mg/kg body weight/day.

In view of the maximum oral doses of PS 80 or 20 in authorised medicinal products the oral exposure of PS 80 by oral formulations is estimated to be far below ADI.

In conclusion, a threshold for oral administration of polysorbates as excipients is not considered meaningful.

However, as it is known that polysorbate 80 increases gastrointestinal absorption of other drugs (see 3.3.) this potential PK interaction should be taken into account in SmPC/PIL.

Parenteral exposure

Hypersensitivity reactions including anaphylactic shock have been observed after IV administration. Therefore, a respective warning of allergic reactions at threshold zero is proposed.

In contrast to the oral route, after intravenous administration intact polysorbates enter the bloodstream. But even after IV administration polysorbates are rapidly cleared from plasma (half-life < 1h) probably due to hydrolysis by blood lipases and esterases. The resulting fatty acid moieties are probably catabolised following the normal metabolic pathways of unsaturated fatty acids whereas the polyoxyethylene moiety is mainly excreted unchanged by the kidney (see chapters 2.3 and 3).

It has been known for a long time that polysorbate 80 can enhance the uptake of drugs into the brain. Enhancement of brain uptake of other drugs is observed after i.v. doses of 3.2 mg/kg/d in mice and 20 mg/kg in rats (Azmin et al. 1985, Calvo et al. 2001). These doses correspond to a human equivalent dose (HED) of 0.3 mg/kg/d and 3.3 mg/kg, respectively (divided by the allometric factor 12 for mice or 6 for rats). Since these were the lowest doses tested in the studies, the effect might have also occurred at lower doses.

Induction of endocytosis and/or transcytosis of the coated particles is favored as underlying uptake mechanism by polysorbate 80, but also membrane lipid solubilisation, opening of tight junctions or inactivation of the P-glycoprotein efflux pump could contribute to the effect. This ability is utilised in the coating of nanoparticles with PS 80 for drug delivery to the brain (Kreuter 2013). However, simple addition of polysorbate 80 surfactant solution to doxorubicine was totally inefficient compared to

coated nanoparticles. Nevertheless, this ability of polysorbates constitutes a potential interaction with drug substances which should be taken into account during benefit-risk evaluation of current and new parenteral products containing polysorbates.

Evidence for a cardiotoxic/torsadogenic potential of polysorbates comes from in vitro data on hERG current inhibition as well as from preclinical data showing an increase in effective refractory period (ERP) in guinea-pig cardiac preparations and in vivo in dogs (see chapter 2.1.3). Block of I_{Kr} (hERG channels) by polysorbate 80 might explain the observation of increased ventricular ERP in the dog after i.v. administration of 20 mg/kg polysorbate 80 (Torres-Arraut et al. 1984 [100]). Some data indicate that polysorbate 80 is a "multi-ion channel blocker" in the heart inducing cardiac electrophysiological effects not only via block of I_{Kr}. The electrophysiological studies performed in guinea-pig cardiac preparations (Batey et al. 1997 [7]) and in-vivo in dogs (Torres-Arnault et al. 1984 [100]) were published several years ago and, therefore, the methods do not seem to be completely state-of-the-art of the year 2017.

Precautionary safety limits with regard to cardiotoxicity could be approximated from in vitro IC50 of PS 80 for inhibition of hERG currents which is reported to be 0.2 mg/ml (0.02%; IC50 of PS 20 similar, see 2.1.3). According to Redfern et al. 2003, a 30-fold margin between free therapeutic plasma concentrations and IC50 values for block of hERG currents appears to be a line of demarcation between the majority of drugs associated with Torsades de Pointes (TdP) arrhythmias and those which are not. Division of IC50 by 30 results in a plasma concentration of 0.007 mg/ml (0.0007%) polysorbate 80 which should not be exceeded in vivo. Following this, an IV bolus dose of 0.35 and 0.7 mg/kg for adults and infants, respectively can be regarded as safe, because it will not exceed this initial plasma concentration. This derivation is valid for bolus injections only. When infusions are administered more slowly (e.g. over 1h), much lower PS levels were measured at the end of infusion than expected from calculation for a bolus dose: humans receiving very high polysorbate 80 doses of 3–4.5 g (50-75 mg/kg) via Taxotere® infusions (1h) yielded end of infusion plasma concentrations of about 0.01% (Webster et al. 1997 [106]). These are tenfold lower than expected from a 3 g bolus dose distributing in 3 L Plasma (0.1%). This is in line with the rapid plasma clearance of PS 80 observed in adults (7.7 L/h, see chapter 3.1.2).

For cases of administration as continuous infusion, alternatively, an infusion rate (Rinf) can be estimated which would not exceed a steady state concentration in plasma (Css) of 0.007 mg/ml (using equation Rinf = CL*Css). From this a "safe" continuous infusion rate of < 0.015 mg/kg/min for an adult (60 kg) is calculated. This corresponds to a safe cumulative dose of 21 mg/kg/d when given as continuous infusion. With respect to slower metabolism of polysorbates, i.e. lower CL/kg, expected in infants compared to adults (see 3.2.), an additional safety factor for infants could be discussed.

In vivo bolus doses ≥ 10 mg/kg of PS 80 alone lead to depression of the cardiac conduction (prolongation of the sinus node recovery time, depressed AV-nodal function and increased atrial effective refractory period (ERP)) and hypotension in dogs (Torres-Arraut et al., 1984 [100], Masini et al., 1985 [65]). The authors concluded that polysorbate 80 is a potent depressant of the cardiac conduction system in the dog and its electro physiologic effects are similar to those of amiodarone. Infusion rate was 2 mg/kg/min (over 5 min) in the Masini study (Masini et al., 1985 [65]) when hypotension/Histamine release was observed. Recent preclinical data in dogs (Cushing et al., 2009) unequivocally confirmed that the hypotensive effects of commercial amiodarone IV result from the co-solvents (PS 80 and benzyl alcohol) in the formulation. Polysorbate 80 exposure was even lower in this study (4.3 mg/kg, infusion rate: 0.43 mg/kg/min). It cannot be excluded that benzyl alcohol also contributed to the hypotensive response due to its negative inotropic effects (Yasaka et al. 1979, see Cushing et al., 2009).

There is no evidence so far for depression of cardiac conduction from clinical data in humans. Some authors speculated that the reported cases of death of several neonates after parenteral administration of a vitamin-E preparation (E-ferol) containing 9% polysorbate 80 and 1% polysorbate 20 might be due to block of I_{Kr} by polysorbate 80 (Pesce and McKean 1989). However, cases occurred at much higher cumulative dose levels (> 80 mg/kg/day) where hepatotoxicity is predominant (see below).

In human adults a significant hemodynamic effect (short duration vasoplegia, left ventricular systolic pressure decreased) was observed after amiodarone IV injection (Cordarone®) compared to a formulation without polysorbate and benzyl alcohol (Munoz et al. 1988). PS dose (10 mg/kg) was given as a 3 min bolus (rate for PS 80: 3.33 mg/kg/min). Effects occurred immediately during the injection and were short-lived. The authors concluded that "the risk of severe hypotension after i.v. Cordarone IV® can be largely avoided by using a slower rate of infusion". (Current dose recommendation for the loading rapid infusion of Cordarone I.V. (US label): 150 mg amiodarone over the first 10 minutes, which equals to a PS 80 dose of 4.3 mg/kg at a rate of 0.43 mg/kg/min). This is in contrast to dosage recommendations for amiodarone products in the EU (e.g. Amiodaron-ratiopharm®, DE) which include a bolus injection of 5 mg/kg (corresponding to 10 mg/kg PS 80) over ≥3 min).

Of note, after an accidental high total PS 80 dose (100 mg/kg) infused at a similar rate of 3.3 mg/kg/min (via amiodarone IV), a cardiogenic shock was observed in a 4-day-old newborn (Masi et al. 2009).

Maximum plasma concentration of PS in humans after IV injection of a bolus injection of 10 mg/kg is roughly estimated to be 0.1– 0.2 mg/ml (plasma volume of 60 ml/kg assumed). This is in the range of IC50 at hERG channels (0.2 mg/ml) and of the concentrations needed to elicit cytotoxic effects of PS 80 on cells in vitro (0.05 mg/ml). These considerations might add mechanistically support for a 10 mg/kg bolus dose as a plausible safety limit.

Support for the safe short term exposure of PS 80 < 10 mg/kg per day in infants and neonates comes from a small PK and safety study in infants and neonates (Cohen-Wolkowiez et al 2011): Anidulafungin has been given to neonates (8) and infants (9) at 3 mg/kg/day loading dose (day 1) and subsequently at 1.5 mg/kg/day for 3–5 days (according to the posology of Ecalta®). These doses correspond to PS 80 doses of 7.7 mg/kg/day on Day 1 and 3.8 mg/kg/day over 3–5 days. PS infusion rates (over 60 min) were 0.064– 0.13 mg/kg/min. There were no product related serious adverse effects. From this a safe short-term exposure limit for PS 80 of ≤ 4-8 mg/kg/d given at an infusion rate of < 0.15 mg/kg/min could cautiously be deduced for infants and neonates > 1 months of age. (Of note, non-serious events included elevation of liver enzymes).

MVI paediatric is used in the US and has been used over a long period also in Europe even though not authorised without any apparent safety issues. The cumulative dose of polysorbate 80 in MVI paediatric is very high, maximally 32.5 mg/kg/day in a 1 kg neonate/infant given as an infusion over 24 hours. The safe use of this product is apparently contradictive to the high cumulative daily dose. However, it is given as a continuous infusion, and at the maximal dose the hourly rate is 1.35 mg/kg/h for a 1 kg neonate. This equals to a rate of 0.023 mg/kg/min which is markedly below the injection rate of PS 80 via amiodarone bolus injection in adults leading to hemodynamic effects (3.33 mg/kg/min) and below the rate of 0.13 mg/kg/min considered as safe in neonates and infants from the Cohen-study (see above). This could well explain why this product is safely used and further supports that rate of injection (peak exposure, C_{max}) might be more important than the cumulative dose, at least for cardiovascular/cardio toxic effects.

In summary, the hemodynamic (and perhaps also the potential cardio toxic) effects appear to be rather related to the infusion rate (peak exposure) than to the total dose (cumulative exposure). An infusion rate of 0.015 mg/kg/min is theoretically considered as safe (from IC50 of hERG inhibition), a 10-fold higher rate of 0.06-0.13 mg/kg/min (up to a total dose of 4-8 mg/kg/day) has been proven to be safe in infants and neonates (anidulafungin, Ecalta®, Cohen-Study, see above). On the other hand, short infusions at rates of 0.43 – 2 mg/kg/min lead to hypotension/histamine release in dogs (Cushing et al. 2009, Masini et al. 1985), and a rate of 3.3 mg/kg/min (for 3 min) was shown to be associated with hemodynamic effects in adults and (for 30 min) with a cardiogenic shock in a 4 day old new-born (Munoz et al. 1988 [70], Masi et al. 2009 [64]).

From the totality of preclinical and clinical data a threshold of 10 mg/kg (given as bolus dose) is proposed. It should trigger a warning regarding cardiovascular effects (hypotension/cardiac depression), since bolus doses above that level were associated with such effects in humans and dogs.

In addition, it is concluded that further (pre-clinical and) clinical electrophysiological studies are warranted to investigate the torsadogenic potential of polysorbate 80 in detail (according to the ICH S7B and E14 Guidelines, e.g. measurement of action potential parameters in isolated cardiac preparations, measurement of proarrhythmic effects in isolated cardiac preparations, evaluation of polysorbate 80 regarding the TRIAD concept; a “thorough QT study” in humans according to the E14 Guideline). Due to the potential effect on hERG channels by polysorbates synergistic effects might occur after administration of polysorbate 80 in combination with other hERG channel blockers. Therefore, a warning on the risk of concomitant use of medications that prolong the QT/QTc interval should be considered for the SmPC/PIL of all products containing polysorbates above this threshold.

Furthermore, a parenteral threshold triggering a warning of hepatotoxicity is deemed necessary. From the E-Ferol tragedy a cumulative dose limit of 80 mg/kg/day (corresponding to an infusion rate of 0.055 mg/kg/min when applied as continuous infusion) for severe hepatotoxicity in premature infants was deduced, because no cases (defined as illness following the clinical diagnosis of ascites or occurrence of at least two clinical laboratory abnormalities) occurred at doses that were below 20 units of alfa-tocoferol, which corresponded to doses below 72 mg/kg/day and 8 mg/kg/day for PS 80 and PS 20, correspondingly (Martone et al., 1986 [62]). The toxicities reported were after infusions administered continuously over 24 hours, and they occurred after a longer time of infusion or even after administration has been stopped. This suggests that cumulative doses rather than short term peak exposure levels appear to be relevant for hepatotoxicity. But it is still unclear whether toxicities might be related to peak concentrations or cumulative concentrations, and whether the toxicity might depend on rate of administration.

Case reports in adults at exposures below 80 mg/kg/d may indicate an earlier onset of signs of hepatotoxicity: 35-40 mg/kg were calculated as the cumulative PS dose within 24 h identified in case reports of hepatotoxicity in adults after Amiodarone IV, e.g. showing abrupt elevation of liver enzymes (see 4.1). Such case reports are confounded by the fact that amiodarone itself is a hepatotoxic agent. However, the observation that oral amiodarone administrations in such patients do not result in additional liver toxicity supports the association with the intravenous exposure of the excipient.

In conclusion, a lower threshold of 35 mg/kg/d for all age groups is suggested to trigger a warning for elevation of liver enzymes. This threshold would be supported by the fact that it is above the exposure expected from MVI paediatrics (maximally 33 mg/kg/day in a 1 kg neonate/infant) which has been used over a long period in the US (and also in Europe) without any apparent safety issues. However, it would be below the expected PS exposure from Taxotere® (55 mg/kg). As with amiodarone, docetaxel itself is potentially hepatotoxic, it is thus not possible to attribute cases of severe hepatotoxicity after docetaxel solely to polysorbate 80. However, in recent studies comparing Taxotere® with new

polysorbate-free formulations, hepatotoxicity was not among the differences identified (Tagawa et al., 2017 [93]).

Impact of the proposals on the labelling of parenteral medicinal products in EU:

Small molecules

Anidulafungin (Ecalta®): PS dose: 8.5 mg/kg/d (contin. Infusion; above zero threshold: labelling of content/allergy)

Amiodarone (e.g. Amiodaron-ratiopharm®, DE): PS exposure by bolus injection dose: 10 mg/kg (above first and second threshold: labelling of “cardiovascular effects, e.g. hypotension”)

Docetaxel (Taxotere®): PS exposure by single dose: 55 mg/kg (above all thresholds; labelling: content/allergy, cardiovascular effects, liver enzymes)

Proteins

PS exposure by administration of therapeutic proteins is low (< 1 mg/dose, < 0.25 mg/kg). The bolus doses lie below all thresholds apart from zero. Even if administered as slow infusion the infusion rates are expected to be below 0.15 mg/kg/min which could be regarded as safe even in neonates and infants. E.g. Herceptin® loading dose (including PS exposure dose of 1 mg/kg) is given as a 90 min infusion. Thus, infusion rate is calculated as 0.01 mg/kg/min.

Vaccines

The highest PS content is 0.75 mg/vaccine dose which is equivalent to 0.75/60 kg = 0.0125 mg/kg in adults (worst case: 16 year old/30 kg→ 0.025 mg/kg). The highest content in a vaccine authorised for use in infants/neonates is 0.1 mg/D which corresponds to 0.03 mg/kg in a 3 kg infant. All derived dose levels of PS 80 would be far below the lowest threshold bolus dose above zero proposed.

A higher PS content per vaccine dose is observed in vaccines containing oil adjuvants. The seasonal influenza vaccine Fluad is authorised for elderly adults (> 65 years) only. The PS dose of 1.175 mg/vaccine dose is equivalent to about 0.02 mg/kg for a 60 kg person. This is also well below the first proposed threshold above zero.

This is considered appropriate as it is in line with the absence of any signal of cardiotoxicity/hepatatoxicity after vaccine exposure from epidemiology or pharmacovigilance.

Further support is derived from the following considerations: Considering a worst case scenario of complete systemic availability of the total dose of PS 80 administered with one vaccine dose (max. dose assumed: 0.75 mg), maximum plasma concentrations of intact PS 80 of about 0.25 µg/ml in adults (70 kg) or 1.5 µg/ml in children (10 kg) or 3 µg/ml in neonates (3 kg) can be conservatively estimated (assuming intravenous injection of the total dose into plasma volumes of 3 l, 0.5 l and 0.25 l, respectively). These concentrations are not expected to have any effect, they are even below the precautionary limit of 0.0007 mg/ml (7 µg/ml) with regard to potential QT prolongation (see above) and far below concentrations eliciting membrane/cytotoxic effects on cells in vitro (CC50 for cytotoxicity in vitro: 48 µg/ml; see PD chapter, table 2 at the end of section 2.1).

Even for Pandemrix®, which contained a higher PS content in its emulsion adjuvants (4.85 mg/D), the estimated worst case maximum plasma concentrations after one vaccine dose (assuming sudden 100% bioavailability) would calculate as low as 1.6, 9.7, and 19.4 µg/ml in adults, children, and neonates, respectively.

With respect to vaccine-induced narcolepsy, it is noted that there is scientific evidence indicating that a special antigen ingredient is more likely to be causative for the development of narcolepsy than the Polysorbate 80 containing adjuvant (Ahmed and Steinman, 2017 [2]).

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