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Systemic availability of lipophilic organic UV filters through dermal sunscreen exposure

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ABSTRACT

Background: Chemical UV filters are common components in sunscreens and cosmetic products and used to protect the skin against harmful effects of sunlight like sunburn. However, the effectiveness of sunscreens in the prevention of skin cancer is in some parts still controversial. Meanwhile, questions about negative effects of the chemical UV filters on human health arise and request an effective risk assessment. Real-life exposure data in humans after application of these products are still rare. Thus, we explored whether and to what extent UV filters are absorbed through the skin into the human body.

Material and methods: Plasma and urine samples from 20 healthy volunteers were collected before, during and after a real-life exposure scenario (1st application: 2 mg/cm^2; 2nd and 3rd (after 2 and 4 h): 1 mg/cm^2 each) using a commercial sunscreen formulation for one day. These samples were analyzed for their content of the currently prominent UV filters octocrylene and avobenzone as well as 2-cyano-3,3-diphenylacrylic acid (CDAA) as the main octocrylene metabolite by using different liquid chromatography electrospray-ionization tandem mass spectrometric procedures.

Results: Following dermal sunscreen exposure, avobenzone, octocrylene and CDAA reached concentrations up to 11 µg/L, 25 µg/L and 1352 µg/L in plasma. In urine detection rates of avobenzone and octocrylene were low while CDAA showed a high detection rate and reached up to 5207 µg/g creatinine. Kinetic models could be fitted for octocrylene and CDAA in plasma and CDAA in urine. Concentration peaks were reached between 10 and 16 h after first application and half-life periods were in the range of 1.5 to 2 days. The lipophilic UV filter octocrylene and its metabolite CDAA showed a much slower elimination than other more hydrophilic UV filters. Concordantly, the metabolite CDAA in particular showed a markedly increased renal excretion over the whole sampling period and indicated high internal exposure to OC.

Discussion: Real-life sunscreen usage leads to considerable bioavailability of organic UV filters and their metabolites which is rarely seen for other environmental exposures. A combined monitoring of the parent compound and its metabolites is important to fully address internal exposure to the UV filter in humans. Considering the kinetic profiles a prolonged systemic release due to depot formation in skin and a potential accumulation through multi-day exposure is presumed. High in-vivo loads call for a critical toxicological assessment of the UV filters and their metabolites.

1. Introduction

UV Filters are widely used throughout the world in cosmetic and personal care products as well as in plastics and industrial products for their UV-absorbing properties. They either protect the human skin against deleterious effects of UV radiation or prevent photodegradation of the products itself (Gago-Ferrero et al., 2012; Manova et al., 2013; Uter et al., 2014; Wang et al., 2016). Two organic UV filters commonly used nowadays are avobenzone (AVO; CAS No. 70356-09-1; molecular weight: 310.4 g/mol) and octocrylene (OC; CAS No. 6197-30-4;

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molecular weight: 361.5 g/mol) (Kerr, 2011; Manova et al., 2013; Uter et al., 2014). Due to their widespread use with continuous anthropogenic input into the ecosystems organic UV filters have been detected in a wide array of biota such as sediments, surface and marine water, fish and mussels and even in marine mammals, thus demonstrating bioaccumulation in the food chain (Balmer et al., 2005; Gago-Ferrero et al., 2012; Baron et al., 2013; Gago-Ferrero et al., 2013; Apel et al., 2018a; Apel et al., 2018b; Vidal-Linan et al., 2018).

Detrimental and adverse effects have not only been described for aquatic organisms (Ozaez et al., 2016b; Campos et al., 2017; Giraldo et al., 2017; Li et al., 2018) but also in mammals and humans. Besides the possible causation of (photoallergic) contact dermatitis in some individuals (de Groot and Roberts, 2014) especially endocrine disrupting effects (Witorsch and Thomas, 2010; CEHOS, 2013; Gilbert et al., 2013; Wang et al., 2016) as well as neurotoxic and metabolic disrupting potential (Ruszkiewicz et al., 2017; Ahn et al., 2019) have been described. However, those assumptions are mostly based on data by in-vitro studies and their in-vivo relevance remains to be clarified. For example, in-vitro studies on OC showed affinity to various hormonal receptors or effects attributed to such capacities (Kunz and Fent, 2006; Balazs et al., 2016; Ozaez et al., 2016a; Zhang et al., 2016). However, it is unclear how these receptor interactions translate to a possible adverse outcome in-vivo as a reproductive and developmental toxicity could not be confirmed in rat studies (European Chemicals Agency). Concerning AVO, in-vitro data indicated that a possible endocrine disruption potential might be present but no uterotrophic effect could be demonstrated in-vivo (Schlumpf et al., 2001; Schreurs et al., 2005; CEHOS, 2013). Furthermore, AVO has been suspected to be a human obesogenic chemical as it significantly promoted adipogenesis in in human bone marrow mesenchymal stem cells (Ahn et al., 2019). Health concerns become especially relevant considering that sunscreen products are frequently applied around the world (Mancuso et al., 2017; Seite et al., 2017) including particularly vulnerable or susceptible individuals such as pregnant women and children. The annual production volume of sunscreen has increased significantly lately. In Germany the production volume almost doubled over the last decade (Destatis, 2018).

The application of UV filters directly on the skin needs consideration in human exposure assessment as the dermal exposure is probably the most relevant exposure route of UV filters for humans both in daily life and in outdoor work. In-vitro penetration into the stratum corneum and in some cases even through the skin has been shown (Jiang et al., 1999; Potard et al., 2000; Chatelain et al., 2003; Iannuccelli et al., 2008; Montenegro et al., 2008; Scalia et al., 2011; Montenegro and Puglisi, 2013). Even more importantly, recent studies have reported systemic absorption of organic UV filters in humans after sunscreen application. Under a maximum use scenario concentrations of AVO and OC in plasma exceeded threshold levels set forth by the US Food and Drug Administration (FDA) for waiving nonclinical toxicology studies (Matta et al., 2019). In support of these findings, an increasing excretion of specific OC metabolites in urine after a dermal sunscreen application in one volunteer has been described (Bury et al., 2019) as well as higher background levels in persons who had used sunscreen in the previous 5 days as opposed to nonusers (Bury et al., 2018).

These studies suggest that the currently often used organic UV filters AVO and OC reach the circulatory system in humans after sunscreen exposure and a dermal exposure to those UV filter might contribute substantially to their overall burden in humans. However, reliable data on the extent of dermal penetration and in-vivo fate under real-life conditions are still limited and need further addressing. Accurate risk assessment needs to be based on sound exposure data. Therefore, the study objective was to determine the systemic availability and renal elimination of AVO, OC and the main OC metabolite after a whole body dermal sunscreen exposure under real-life conditions in humans. A particular focus was placed upon concurrent monitoring of both parent compound and metabolite as well as determination in plasma and urine samples.

2. Materials & methods

2.1. Study design

A commercial sunscreen lotion[2] with a sun protection factor (SPF) of 30 containing three currently often used organic UV filters and titanium dioxide as active ingredients was selected for our study. Besides AVO and OC the sunscreen also contained 2-ethylhexyl-salicylate (EHS) as a more hydrophilic organic UV filter whose urinary excretion has been investigated as well and is reported elsewhere (Hiller et al., 2019). The concerted study protocol is described in detail below.

For the real-life exposure all volunteers spent one day outside dressed in bathing clothes in a separated, partly shaded park area of the university on a summer day in July 2017. There were no requirements for the participants concerning the stay in the sun or shade with both options readily available throughout the day. The weather was in parts lightly overcast in the morning but mostly sunny (9 h of sunshine according to the meteorological data for the day), reflecting a typical German summer day. Temperatures reached a maximum of 27.4 °C in the afternoon with relative ambient humidity being around 50%. Food and beverages ad libitum were provided by the organizing team during the exposure period. Food items were selected with a preferred usage of cutlery in mind to minimize hand-mouth contamination and participants were instructed to avoid hand-mouth contamination.

The individual amount of sunscreen was calculated using the body surface area (BSA) and a skin dose of 2 mg sunscreen per cm^2 BSA. This is the testing dose used to obtain the stated SPF of a sunscreen product according to ISO 24444:2010 test guideline (ISO, 2010). Therefore, the volunteers received between 30.8 and 41.2 g of sunscreen for the initial application. After the first application the volunteers received half of the initial amount each for a second and a third dosing of sunscreen 2 and 4 h after the initial application to account for the recommended reapplication of sunscreen (Diffey, 2001; Petersen and Wulf, 2014; Matta et al., 2019).

After collecting baseline samples (t_0) all subjects were given their first individualized sunscreen portion and asked to apply all of it by themselves for the start of the exposure period. Subjects wore bikinis or swim trunks and applied the sunscreen to the uncovered skin, i.e. all areas outside the normal swimwear but sparing lips, eyes, hairy scalp and plantae. Therefore the amount of body surface covered with sunscreen amounted to approximately 75 to 80% gauging by the rule-of-nines. The subjects stayed outside at the university's premises until 8.5 h after initial application. Afterwards, the subjects were asked to go home and shower promptly using a standardized shower gel to end external sunscreen exposure after 9 h.

Blood samples were drawn during the exposure period after 1.75, 3.75, 5.75 and 7.75 h as well as after 24, 48 and 72 h. The blood samples were centrifuged and plasma was frozen at −20 °C within a day until analysis. All excreted urine was collected in separate portions with a clean polypropylene measuring cup containing up to 1 L and marked every 10 mL to obtain complete separate urine voids during the first 24 h. One-hour-intervals were requested during the on-site exposure period starting 30 min after the first application. Afterwards the collection of complete fractioned urine samples was continued by the study subjects without time restrictions for the remaining 15 h. For follow-up, complete morning urine samples were requested for another

[2] Ingredients: Aqua, Octocrylene, Alcohol, Glycerin, C12–15 Alkyl Benzoate, Ethylhexyl Salicylate, Titanium Dioxide (Nano), Butyl Methoxydibenzoylmethane, Propylheptyl Caprylate, Stearyl Dimethicone, VP/Hexadecene Copolymer, Panthenol, Butyrospermum Parkii Butter, Parfum, Tocopheryl Acetate, Silica, Ethylhexylglycerin, Acrylates/C10–30 Alkyl Acrylate Crosspolymer, Carbomer, Sodium Hydroxide, Xanthan Gum, Disodium EDTA, Dimethicone, Linalool, Limonene, Benzyl Salicylate, Eugenol, Citronellol, Coumarin, Tocopherol.

6 days. The excreted volume and the sample time of each urine sample were recorded by the participants on a documentation form. An aliquot was immediately bottled in a sterile polypropylene 100 mL container and frozen until analysis. An exact timeline protocol with sampling time points as well as the exposure sequence can be found in the supplementary material (Fig. S3).

The study design, sample collection and data processing were approved by the local ethics committee of the University of Erlangen-Nürnberg (Re.-No. 122_17B; 17.05.2017). Informed written consent was obtained from each participant prior to inclusion.

2.2. Study collective

The following exclusion criteria were defined for the recruitment of the study collective to minimize concurrent exposure and health risks:

- Age of < 18 or > 50 years
- current or previous malignant or semi-malignant skin tumor
- autoimmune or genetic disease with elevated UV sensitivity thereof
- use of UV sensitizing/phototoxic medication
- known allergy against sunscreen products
- known polymorphic light eruption (so-called “sun allergy”)
- pregnant or lactating women
- use of sunscreen products or cosmetic products containing chemical UV filters in the week before and after the exposure
- use of insect repellents during and in the 3 days before and after the exposure

28 volunteers (n = 14 men; n = 14 women) who complied with the above stated criteria were recruited primarily from the students of the University of Erlangen-Nürnberg, paying attention to a balanced sex distribution.

To calculate the individual BSA with the formula of DuBois (DuBois and DuBois, 1989) their anthropometric data were measured. Furthermore skin blemishes such as scratches were clinically inspected and documented as a possible factor of influence regarding skin penetration. Skin and eye color as well as the subject's reports of how their skin responds to the sun were assessed to determine the skin pigmentation type according to Fitzpatrick (1988).

The consumption of beverages was not restricted during the exposure period due to the warm weather conditions. Therefore, sometimes a high overall urinary excretion was observed. To ensure an acceptable sample quality eight candidates with > 4000 mL urine excretion during the first 8.5 h after first application or > 5000 mL within 24 h, i.e. highly unphysiological parameters, were excluded before chemical analysis of the samples.

2.3. Chemical materials and analysis

The UV filter concentrations of the chosen sunscreen were 2.34% AVO and 10.85% OC, determined by the Bavarian State Office for Health and Food Safety. The two lipophilic UV filters OC and AVO were selected as the primary analytical targets. As recent research identified specific metabolites for OC, and 2-cyano-3,3-diphenylacrylic acid (CDAA) as one of those could be identified as the main metabolite representing 45% of the OC dose after oral administration (Bury et al., 2018, 2019), CDAA was included in the analyses. The parent compounds and the metabolite were analyzed in plasma by liquid chromatography electrospray-ionization tandem mass spectrometry (LC–MS/MS) and in urine by using two-dimensional LC/LC–MS/MS procedures as described elsewhere (Klotz et al., 2019). The limits of detection (LOD) were 1.1 µg/L plasma and 1.5 µg/L urine for AVO, 1.6 µg/L plasma and 1.44 µg/L urine for OC and 6.5 µg/L plasma and 0.45 µg/L urine for CDAA. Creatinine content of the urine samples was determined photometrically according to the Jaffe's method (Larsen, 1972).

2.4. Data processing and statistical analysis

The initial results of the analysis were obtained in µg/L for both matrices. However, to account for different urinary dilutions, absolute urine concentrations were subsequently converted to a creatinine-related concentration. Additionally, the absolute urine concentration in µg/L was multiplied with the excreted sample volume to calculate the total cumulative excreted amount of the analytes during the fractioned urine sampling period, therefore arriving at a creatinine and dilution independent result for the first 24 h.

Plasma samples below the LOD were replaced by half the LOD. Urine samples with concentrations below the LOD were factored in the kinetic calculations with 1.25 µg AVO/g, 1.22 µg OC/g and 0.23 µg CDAA/g creatinine.

The relative renal recovery (RRR) was calculated by dividing the cumulative excretion amount in the first 24 h by the amount of analyte applied by each individual.

Plasma and urine concentrations as well as the cumulative excreted amounts were plotted as a function of time using ggplot2 graphics (Wickham, 2016). Mean curves were fitted using generalized nonlinear least-squares, accounting for heteroscedasticity via the power variance function and correlated errors via a continuous-time AR(1) structure for repeated measurements (Pinheiro and Bates, 2000). A log-normal shape was assumed for urinary and blood concentration kinetics, whereas a log-logistic growth curve was fitted to the cumulative excretion amounts. Confidence intervals for fitted curves and derived statistics were computed by parametric bootstrap, including for the peak time and concentration as well as for the partial area under the curve (AUC) above baseline to estimate the relative excreted fraction over 24 h.

Plasma and excretion terminal half-lives ($t_{1/2}$) were calculated with the formula $t_{1/2} = \ln 2 / |k_{el}|$ using the slope (k_{el}) of the linear regression between ln-transformed concentrations and times assuming a first-order elimination process as described previously (Göen et al., 2016). k_{el} is defined as the elimination rate constant. Due to depot formulation this half-life is not an elimination half-life, but a terminal half-life. A mixed-effects model with individual-specific deviations from the global intercept and slope was used to account for repeated measurements.

Rank-based tests for independent samples (Mann-Whitney-U or in case of more than two levels Kruskal-Wallis) were used to explore possible influence factors on bioavailability and excretion rates. Associations between plasma and urine concentrations were analyzed by linear regression analysis using Pearson correlation.

IBM® SPSS® Statistics Version 25, OriginLab Origin® 2018b and the statistical programming environment R 3.5.2, R Core Team, 2018, R Foundation for Statistical Computing, Vienna, Austria. (www.R-project.org/) were used for statistical analysis.

3. Results & discussion

Twenty-eight volunteers (n = 14 men; n = 14 women) were initially recruited, however, as described in the study protocol, eight individuals were excluded from analytical analysis on account of their high excreted urine volumes. With 9 women and 11 men included, the final study collective had a fairly balanced sex distribution. The subject's characteristics showed a narrow age span but considerably different anthropometrics (Table 1). Skin type I according to Fitzpatrick (1988) was seen in 5%, skin type II in 25%, skin type III in 60% and skin type IV in 10%.

For all analytes the baseline load in plasma was below or close to the LOD, especially AVO was not detectable at baseline at all. However, systemic availability of all analytes increased following topical sunscreen application (Fig. 1a–c). AVO could not be detected in plasma in three subjects at any time. For OC and CDAA the data in plasma were sufficient to fit an overall kinetic model. The peak concentrations of OC and CDAA in plasma were reached 10.0 h and 14.5 h, respectively, after

Table 1
Subject characteristics and applied amount of test compounds.

	Mean	SD	Min	Median	Max
Age [year]	24.5	2.6	19	24	31
Height [m]	1.73	0.08	1.56	1.74	1.88
Weight [kg]	70.5	10.6	54.1	70.1	93.3
Body surface area [m^2]	1.83	0.16	1.58	1.85	2.06
Sunscreen applied [g]	73.1	6.4	63.2	73.8	82.4
AVO applied [g]	1.7	0.2	1.5	1.7	1.9
OC applied [g]	7.9	0.7	6.9	8.0	8.9
Total creatinine excretion over first 24 h [mg]	1485	389	768	1502	2257

SD = standard deviation; Min = minimum, Max = maximum.

the first dermal application. Especially for CDAA the 72 h plasma levels were still markedly higher than baseline showing prolonged systemic availability after dermal exposure. Also its parent compound OC showed a systemic occurrence over the whole assessment period. Based on the kinetic models plasma elimination rate constants (k_{el}) of 0.016 and 0.019 h^{-1} and terminal plasma half-lives ($t_{1/2}$) of 44 and 36 h were calculated for the 1st phase period from 24 to 72 h after first application (Table 2). For AVO only the descriptive statistics for each sampling point could be compared and yielded the highest plasma median at 7.75 h after the first application. The individual peak concentrations of AVO were reached within the time frame of 1.75 to 24 h. Considering the available literature data for AVO concentrations in plasma and the LOD of our analytical method of 1.1 µg/L, it is not surprising that we could not detect AVO in the samples of three subjects at any time as the lowest observed AVO concentrations on the first day of a maximum use trial of different sunscreens formulations laid between 0.4 and 1.0 µg/L (Matta et al., 2019).

Complete 24 h urine sampling over the first observation period was proven by calculating the cumulative creatinine excretion over 24 h (Table 1) and comparing that to the expected sex and weight specific reference value (Forni Ogna et al., 2015) . Complete sampling was assumed when the cumulative creatinine excretion was above the respective lower reference value. This was achieved by all subjects. The male volunteers showed a significantly ($p < 0.001$) higher creatinine excretion per day (MD: 1737 mg; Min–Max: 1349–2257 mg) than women (MD: 1155 mg; Min–Max: 768–1557 mg).

Neither AVO nor OC was detected in urine before the sunscreen exposure, while low concentrations of CDAA were found in baseline urine. Following sunscreen application, urinary excretion of the unmetabolized UV filters as well as CDAA was demonstrated. AVO and OC were detected in < 20% of the samples and were mostly close to the detection limits of our methods. Additionally, even in cases in whom it was possible to quantify AVO and OC, respectively, intra-individual variability between the sampling points was high. Therefore, the identification of consistent excretion profiles over time or calculation of the cumulative excreted amount was not possible for both parent compounds. Nonetheless, the vast majority of samples where AVO and OC could be quantified were collected within the first 24 h following dermal application (supplementary material Fig. S4). This timeframe of quantifiable excretion coincided with the plasma availability and indicates reasonable resorption and elimination timelines.

However, the OC metabolite CDAA showed a high urinary excretion after sunscreen application and allowed the derivation of urinary elimination kinetics, the calculation of the total cumulative excreted amount over the first 24 h as well as the relative 24 h excretion fraction (Fig. 2, Table 3). Following topical sunscreen application, a progressive increase of CDAA excretion was found until the maximum urinary concentrations were reached around 16 h after the first application. Similar to the prolonged plasma kinetics, a significantly elevated urinary excretion of CDAA was present over the whole 7 day sampling period (Fig. 2a) and yielded a comparatively long terminal half-life of 38 h (1st phase, period: 22.5–168 h after first application; supplementary material Fig. S2). Comparison of the AUC fractions showed that only 32.9% (95% CI: 30.9–35.0) of the total 7 day urinary CDAA amount was excreted within 24 h after first application and 63.3% (95% CI: 60.7–66.2) within 48 h. Nonetheless, the absolute cumulative CDAA amount excreted over 24 h reached up to 4761 µg which corresponds to 6904 µg octocrylene.

The resorption and elimination timelines of AVO and OC and its metabolite found in our study reflect the typical kinetic profiles expected by the dermal exposure route. An uptake from transdermal absorbed substances into the body may be buffered by a subcutaneous or cutaneous reservoir and lead to delayed but continuous systemic availability and long plasma and urinary elimination periods even after cessation of the external exposure. This has already been described for transdermally absorbed substances (Rom and Markowitz, 2007; Khemiri et al., 2018; Stoeckelhuber et al., 2018). Further support for this assumption can be found in literature comparing the renal excretion kinetics from oral OC dosage (Bury et al., 2018; Bury et al., 2019): After oral administration of OC, the maximum urinary metabolite concentrations for CDAA were found much earlier after 4.2 h and $t_{1/2}$ was 5.7 h for the first phase elimination, and 16 h of the 2nd phase elimination, respectively. The same study group also described an increasing excretion of OC metabolites in the urine of one volunteer after dermal sunscreen application (Bury et al., 2019). In principle, the findings of that study were also consistent with transdermal uptake, even though the recovered amounts and kinetic considerations should be interpreted with caution due to very different exposure conditions (36-times lower OC exposure; shorter exposure duration; missing real-life conditions with heat and sunlight).

The long terminal half-lives of OC and CDAA in plasma and CDAA in urine and the relatively low 24 h and 48 h urinary elimination fractions of CDAA indicate potential accumulation in the human body if exposure is repeated on several successive days – as typical for a holiday or an outdoor-work setting. Even though the data basis for AVO in our study is less robust than that of OC, the observed kinetics and the similar lipophilic chemical structure suggest potential bioaccumulation of AVO, too.

Accordingly, a recently published study on plasma concentrations of active sunscreen ingredients under maximum use conditions demonstrated pharmacokinetic profiles consistent with drug accumulation due to increasing maximum concentrations and AUC over a 4-day exposure period for both AVO and OC (Matta et al., 2019). Although the study focused solely on the parent compounds and the matrix plasma, the findings further support our hypothesis of potential bioaccumulation. An implied serrated increase from day 1 to day 4 could be seen in the plasma profiles of all four tested sunscreen products; although the geometric mean of the time to maximum concentration was found to be around 68–77 h for AVO and 55–75 h for OC, i.e. before the end of the 4-day exposure period, possibly indicating saturatic effects for repeated exposure. The kinetics observed following multi-day exposure are in accordance with the results of our study but raise the question of limiting factors on accumulation due to a saturated intradermal depot formation. When comparing our plasma concentrations to the concentrations found by Matta et al. (2019) the methodical differences from the chosen measures of central tendencies (median vs. geometric mean) should be considered. Nevertheless, the absolute highest plasma concentrations of AVO (9.3 µg/L) and OC (20.4 µg/L) found by Matta et al., 2019 were somewhat below the highest concentrations seen in some subjects in our study (AVO 11.7 µg/L; OC 25.0 µg/L) although our total applied sunscreen amount was lower and exposure duration was shorter. This might be explained by one major limitation of the study by Matta et al. (2019), which the authors also pointed out themselves: The sunscreens were applied without exposure to heat or direct sunlight, i.e., not under typical “real-life” conditions.

Exposure conditions such as solar UV radiation are likely to influence dermal penetration. It has, for instance, been reported that

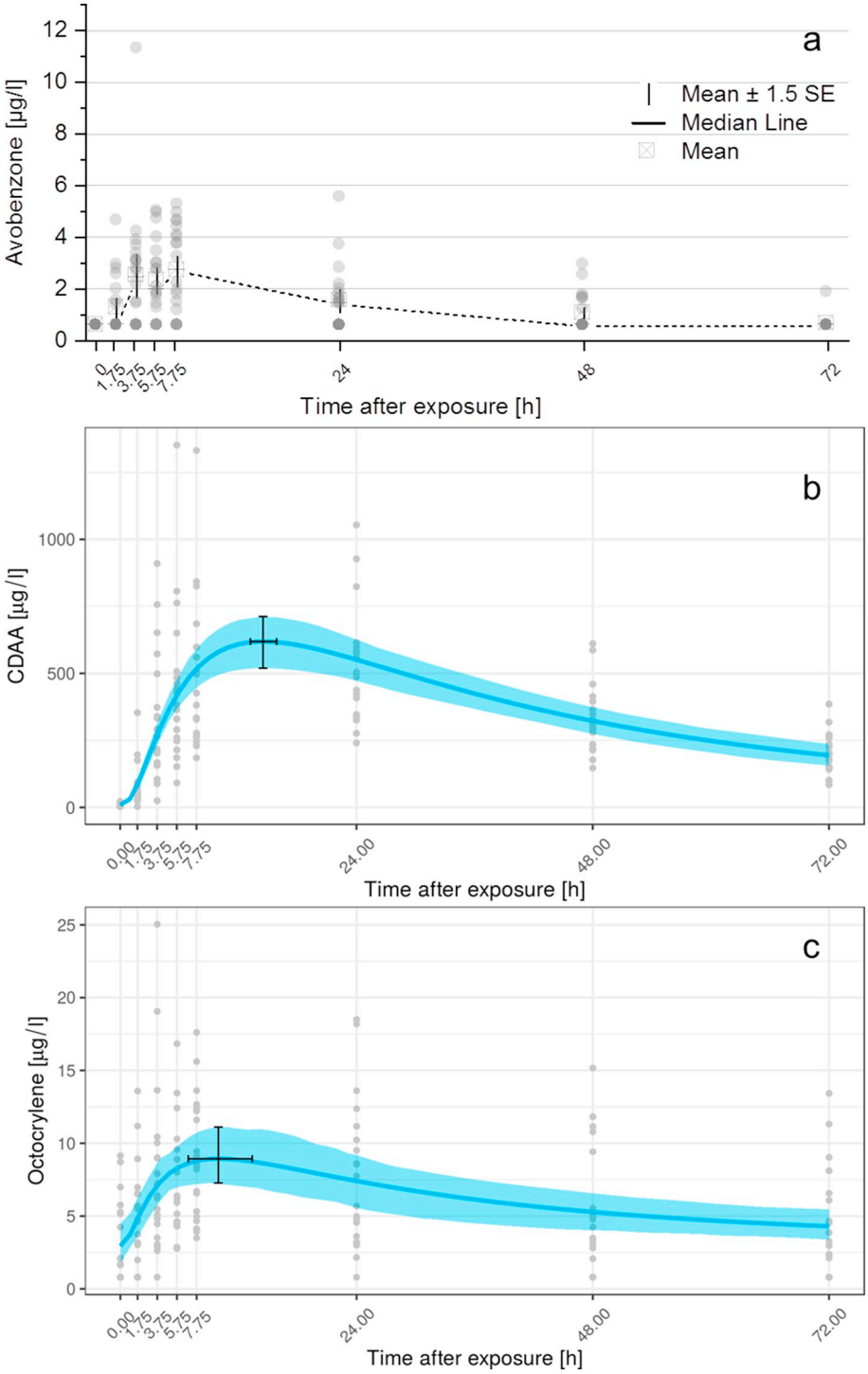

Fig. 1. Pharmacokinetic plasma profiles of avobenzone (a), octocrylene (b) and CDAA (c). For avobenzone the descriptive statistics are depicted, each square and vertical line represents the geometric mean and 1.5 times Standard error of 20 individuals; vertical lines represent the median and are connected through the dotted line. For octocrylene (b) and CDAA (c) the combined log-normal-models with 95% pointwise confidence bands and 95% confidence intervals for the peak time and concentration are depicted. SE = standard error.

simulated sunlight exposure via UV radiation for 16 min increased the penetration of OC into the stratum corneum 1.4-fold and into the epidermis 5.7-fold in diffusion cell experiments (Duracher et al., 2009). This could be due to the irradiation itself interacting with the structure of the substance but also due to direct or indirect temperature effects on the skin and underlying structures such as increased regional blood flow. An augmented skin penetration through increased temperatures had been repeatedly reported (Klemsdal et al., 1992; Emilson et al., 1993). On the other hand, photodegradation of the UV filters in the applied sunscreens due to UV radiation can also be discussed, but seems of more limited importance, as OC was found to be stable under UV exposure in a variety of formulations while AVO showed some degradation (Freitas et al., 2015) but can still be stabilized by other ingredients including OC (Hexsel et al., 2008; Nash and Tanner, 2014). Moreover saturatic effects of a skin depot could hinder a further penetration from multidays exposure with a practically infinite exposure

Table 2
Key figures and toxicokinetic parameters for plasma data.

	AVO		OC		CDAA	
	Median	Max.	Median	Max.	Median	Max.
Concentration at t_0 [µg/L]	< LOD	< LOD	1.7	9.1	7.8	21.9
Max. observed concentration [µg/L]	4.0	11.3	11.7	25.0	570.2	1351.7
Concentration at 72 h [µg/L]	< LOD	1.8	3.1	13.4	198.9	385.5
t_{max} [h]	n.d.		10.0 (95% CI: 6.9–13.4)		14.5 (95% CI: 13.2–15.9)	
k_{el} [h^{-1}]	n.d.		0.016 (95% CI: 0.007–0.025)		0.019 (95% CI: 0.016–0.022)	
Half-life $t_{1/2}$ [h]	n.d.		43.9 (95% CI: 19.0–68.7)		36.1 (95% CI: 31.0–41.2)	
Subjects with analyte detected [%]	85		100		100	
Samples below LOD [%]	57		17		6	
LOD [µg/L]	1.1		1.6		6.5	

n.d. = not determined, LOD = limit of detection, Max. = maximum; t_{max} = time of peak concentration after first application.

dose. Therefore, it is possible that an exposure scenario without real outdoor conditions and sunlight will misjudge the systemic load experienced in real-life in either way.

The overall penetrating amount that becomes systemically available of the two analyzed lipophilic UV filters might also be underestimated in the FDA study by only evaluating the unmetabolized substances. As seen in the present study, considerable quantities of the main OC metabolite CDAA can be found after dermal OC exposure, whereas OC is only measurable in small amounts in the compartments plasma and urine (Tables 2 & 3). Quantitative comparison of AUCs over 72 h from the kinetic plasma models of OC to CDAA showed a ratio of 1:62 (Fig. 3). Nevertheless, a higher availability of OC in other body compartments such as fat tissue or a subcutaneous or cutaneous reservoir may be considered as well and needs addressing in further studies.

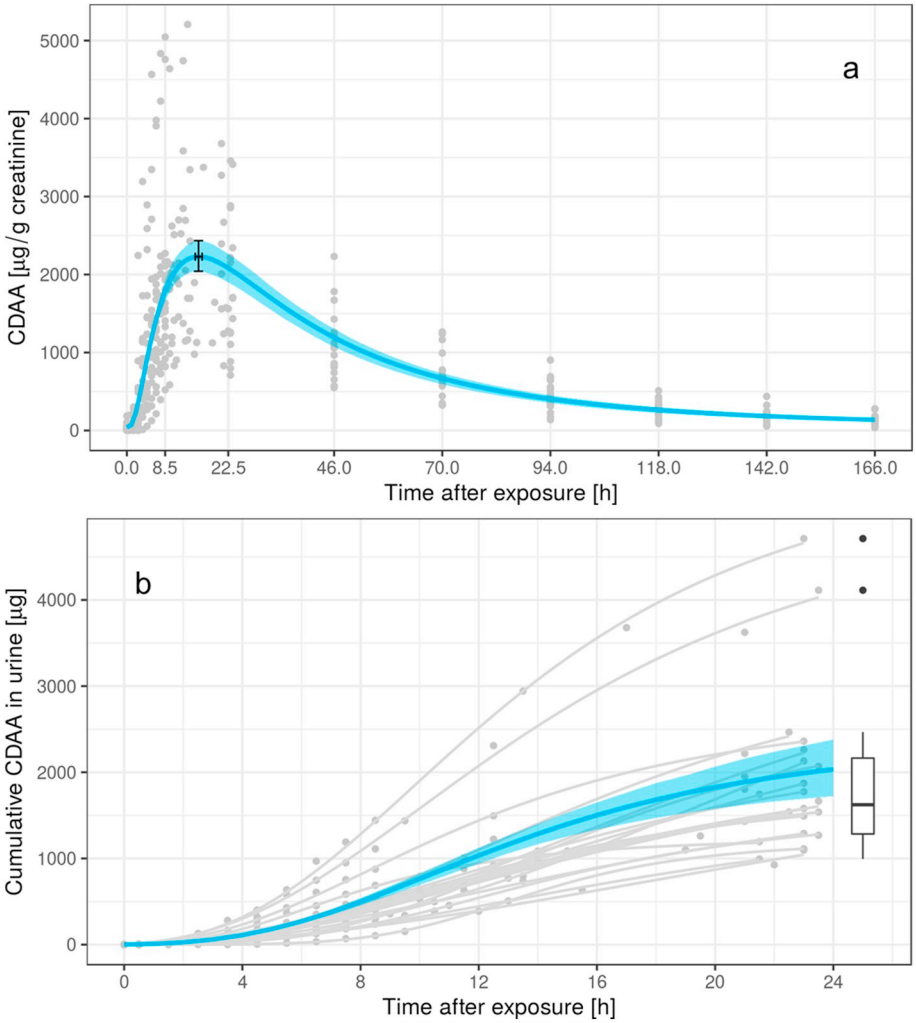

Fig. 2. Time course of the urinary excretion (a) and cumulative excreted amount (b) of CDAA after dermal exposure with combined log-normal-model (a) and combined log-logistic growth model (b) with heteroscedastic and correlated errors. 95% confidence intervals based on parametric bootstrap.

Table 3
Urinary concentrations and kinetics of avobenzone (AVO), octocrylene (OC) and its metabolite CDAA.

	AVO		OC		CDAA	
	Median	Min–Max	Median	Min–Max	Median	Min–Max
Concentration at t_0 [µg/g crea]	< LOD	< LOD	< LOD	< LOD	22.0	0.2–185.4
Max. observed concentration [µg/g crea]	3.4	< LOD–25.2	9.6	< LOD–91.4	2072.4	1127.9–5207.2
Concentration at 166 h [µg/g crea]	< LOD	< LOD	< LOD	< LOD	136.0	36.9–278.9
Cumulative excretion over 24 h [µg], est.	n.d.		n.d.		1630	1087–4761
RRR of applied parent substance [%]	n.d.		n.d.		0.029	0.018–0.086
t_{max} [h]	n.d.		n.d.		15.9 (95% CI: 15.2–16.7)	
k_{el} [h-1]	n.d.		n.d.		0.018 (95% CI: 0.017–0.020)	
Half-life $t_{1/2}$ [h]	n.d.		n.d.		37.7 (95% CI: 35.1–40.4)	
Samples below LOD [%]	87		81		1	
LOD [µg/L]	1.5		1.44		0.45	

n.d. = not determinable; crea = creatinine; LOD = limit of detection; RRR = relative renal recovery; Min = minimum; Max = maximum; t = time point; t_{max} = time of peak concentration after first application; est. = estimated.

Furthermore other elimination routes such as clearing via bile and feces are deceivable as well and would contribute to an underestimation of the penetrated amount.

In this respect, further consideration should be given to the respective concentration profiles and pharmacokinetic data of OC and CDAA in plasma and urine. When their resorption and elimination timelines are simultaneously depicted (Fig. 3) some observations might provide further insight into the metabolization process: The OC metabolite CDAA showed a slightly delayed peak time in plasma (14.5 h vs. 10.0 h, with overlapping confidence intervals) and a slightly shorter terminal half-life (36.1 h vs. 43.9 h) compared to the parent compound. The kinetics of CDAA in plasma were confirmed by similar urinary elimination kinetics with peak time at 15.9 h and half-life of 37.6 h. The congruence of the kinetics of parent compound and metabolite supports the hypothesis that CDAA originates mainly from a hepatic metabolism of systemic available OC, but not from the absorption of CDAA after degradation of OC in the skin.

The urinary CDAA excretion was significantly associated with CDAA plasma levels (Fig. 4; $R^2 = 0.707$). This effect proved to be strongest during the exposure period, while plasma and urinary levels show a higher variability after cessation of exposure (Pearson-correlation at 2 h: $R^2 = 0.882$, $p < 0.001$; at 4 h: $R^2 = 0.766$, $p < 0.001$; at 6 h: $R^2 = 0.616$, $p < 0.001$; at 8 h: $R^2 = 0.643$, $p < 0.001$; at 48 h: $R^2 = 0.128$, $p = 0.122$; at 72 h: $R^2 = 0.213$, $p = 0.04$).

Additionally we evaluated sex and skin type as factors possibly influencing skin penetration. Distinctive inter-individual variations in the recovery rates were found in our collective by the measure of the relative renal recovery (RRR). Therefore, we used the RRR of CDAA (Table 3) as a combined measure for transdermal penetration and systemic metabolism, but the RRR did not differ among sex groups or different skin types.

3.1. Strength and limitations

Our study was performed with the objective to describe and quantify potential systemic availability and urinary excretion of nowadays commonly used organic UV-filters after dermal application of a sunscreen under real-life conditions. Such kinetic data should enable sound exposure assessment to be used in risk assessment. From this background, some limitations need to be discussed.

First, the creatinine levels in some urine samples fell short of the desired range of 0.3–3.0 g/L urine (WHO, 1996). This was probably caused by the high overall beverage consumption due to hot weather. However, we adjusted for diuresis induced variation by using creatinine-based urine concentrations. Additionally, subjects with very high urine volumes in the course of the initial 24 h were excluded from analysis.

Second, a potential oral co-exposure with UV filters through hand-

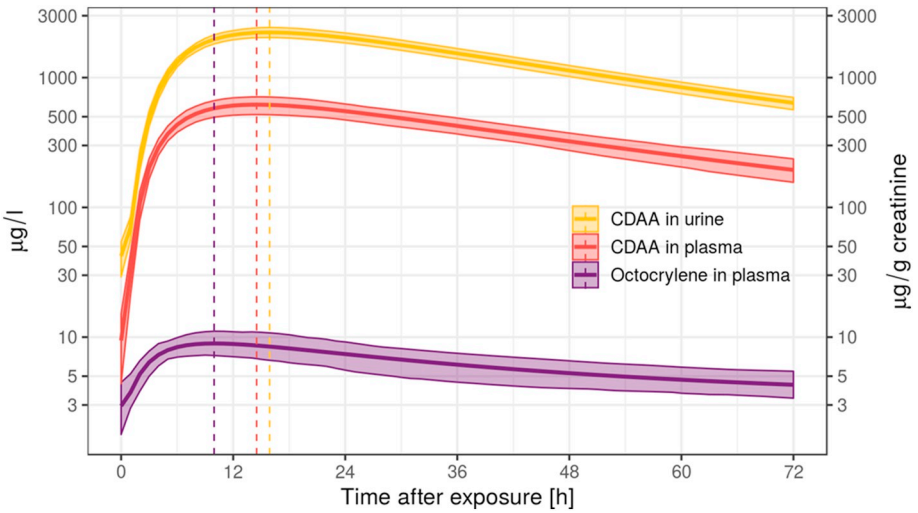

Fig. 3. Comparison of kinetic log-normal plasma models of octocrylene in plasma and its metabolite CDAA in plasma (left y-axis) and urine (right y-axis) on log10-transformed concentration scales. The respective times of peak concentrations are indicated by dotted vertical lines.

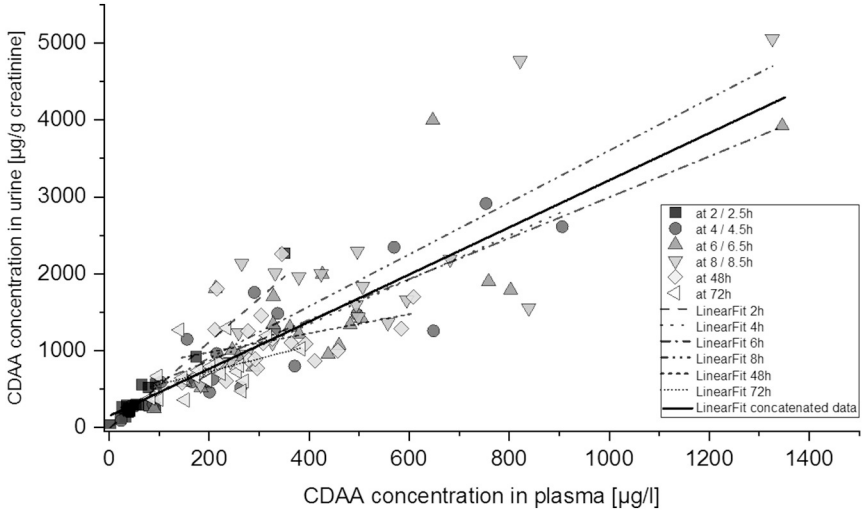

Fig. 4. Comparison of CDAA plasma and urine concentrations at different sampling times.

mouth contamination or swallowing of sunscreen-contaminated sweat cannot be dismissed completely. However, the primary route of systemic exposure was presumably via dermal penetration as we took precautions (sparing of eyes and lips, use of cutlery) as described above. This said, food-consumption during recreational or occupational outdoor activities will inevitably cause some hand-mouth contamination and therefore would even reflect a realistic additional exposure scenario. The plasma and urine profiles found in our study, actually, clearly demonstrate primarily dermal uptake and elimination process and do not suggest a relevant share of oral uptake in comparison with the pharmacokinetic data after oral dosage from previous studies (Bury et al., 2018, 2019).

Third, external exposure with the sunscreen was terminated about 9 h after the first application when the study subjects were requested to take a shower, although the efficiency of the cleaning procedure was not checked. Furthermore, exposure to sunlight (UV) and heat stopped after 8.5 h. Thus, elevated skin temperature and a possibly enhanced regional cutaneous blood flow influencing the drainage from a skin reservoir should exert less effect thereafter. Furthermore, the proximity of the OC and AVO plasma levels to the detection limits of our methods should be critically discussed, although levels showed plausible profiles over time. However, the resulting uncertainty in the calculation approach for the kinetic model and the half-life period for OC in plasma has to be acknowledged. Owing to an increasing number of samples below the LOD in the follow-up (5% after 24 h, 25% after 48 h and 30% after 72 h) and their replacement by one half of the LOD, the calculation of the half-life period faces some uncertainties. Thanks to a better data basis (without any samples under the LOD in the follow-up) this consideration becomes less relevant for CDAA in plasma and urine. Nevertheless, the pharmacokinetic results should be regarded as exploratory. For CDAA the follow-up period did not include the return to baseline levels, therefore comparison of the AUCs slightly overestimates the 24 h fraction in respect to the overall excretion. However, due to the far advanced elimination process after 7 days and the robust elimination data in the respective relevant timeframes thereof, this should not substantially influence the time to peak concentration or the half-life. For AVO in plasma the rather high LOD of our method in comparison with the measured concentrations (highest concentration detected ~10-fold above the LOD) constitutes a limiting factor and led to several subjects without any quantifiable samples. Finally, the pharmacokinetic characteristics (peak time, $t_{1/2}$) were not determined for a single bolus application but for multiple dermal applications over one day. Therefore, $t_{1/2}$ is not a simple unidirectional reflection of the elimination of the systemic burden as a delayed afflux of analytes from (sub) cutaneous reservoirs into the circulatory systems overlaps with its elimination.

Some important strengths should be pointed out as well. Ex-vivo studies may indicate the ability of dermal penetration of UV filters, but do not provide a valid exposure representation, enabling risk assessment, like the present in vivo study. Comparing our data with those of the existing in-vivo studies after dermal application of sunscreens, one important advantage of the present study is the simultaneous determination of the organic UV filter OC along with its metabolite CDAA in plasma and urine. While the study design does not allow acquiring the overall burden fully due to other possible elimination routes (e.g. feces) and possible deposition in body fat tissue, the simultaneous analyte determination in blood and urine allows a better understanding of the metabolism and elimination pathways. Additionally, we mimicked real-life exposure conditions as closely as possible and evaluated the kinetics in a reasonably sized collective. With 20 volunteers, our kinetic profiles are likely to represent the common elimination timeline and allowed a meaningful calculation of time to peak concentration and terminal half-life. The strong correlation between plasma and urine CDAA concentrations during the exposure period supports the reliability of the analytical methods applied. Lastly, by means of our study design with complete fractional urine sampling over the first 24 h, we could determine the total excreted amounts during the first day independent of creatinine or dilution effects greatly enhancing the validity of study results.

4. Conclusion

The results of the present study demonstrate a considerable transdermal penetration of AVO and OC after real-life sunscreen application. The combined monitoring of parent compound and metabolite was crucial for revealing the extent of bioavailability of OC via the dermal route. On the other hand, the high internal load of the specific metabolite CDAA which was found in our study calls for toxicological safety evaluation dedicated to this substance particularly. In-vitro studies focusing on toxic effects of the original active ingredient, that is OC, could miss specific effects of CDAA that might exist, due to a lack of in-vivo metabolism. Similar concerns exist with respect to AVO. Furthermore, additional studies exploring the nature and extent of a possible extrahepatic metabolism, most notably a dermal metabolism, and its comparison with hepatic metabolism pathways would greatly expand existing knowledge. Depot formation and potential bioaccumulation in humans from multiday exposure is very likely for OC and AVO in view of the toxicokinetic data, its extent however is still questionable. A recent study on plasma concentrations of active sunscreen ingredients under maximum use conditions demonstrated pharmacokinetic plasma

profiles consistent with drug accumulation for AVO and OC (Matta et al., 2019). However, as the former study did not include metabolites or urinary excretion, the extent of such potential accumulation remains largely uncharted. It is also conceivable that saturation effects e.g. via intradermal depot formation come into effect, limiting further uptake within a rather short period of time like 2 to 3 days. In conclusion, further studies to extend the knowledge on accumulation effects are needed.

On a final note, although the application of sunscreens is common practice around the world (Mancuso et al., 2017; Seite et al., 2017), the evidence regarding preventive effectiveness and safety of these products is less conclusive (Krause et al., 2012; Bens, 2014; Young et al., 2017; Silva et al., 2018). On the other hand, adverse health effects and potential toxicity of organic UV filters have been discussed controversially repeatedly (Gilbert et al., 2013; de Groot and Roberts, 2014; Wang et al., 2016; Ruszkiewicz et al., 2017; Ahn et al., 2019). So far no limit values for actual human biomonitoring data have been established that could be used in the light of potential toxicological effects. As this study provides further insight into the systemic availability and human load of several organic UV filters following a dermal application, those safety concerns should be further addressed. In particular, as the application of sunscreens or use of organic UV filters in various cosmetic products for photoprotective measures is currently abundant throughout the industrialized world.

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Declaration of Competing Interest

W.U. is a member of the working group “prevention in dermatology” (ADP, www.unserehaut.de) and has received travel reimbursements for attending group meetings.

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Appendix A. Supplementary data

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References

Ahn, S., An, S., Lee, M., Lee, E., Pyo, J.J., Kim, J.H., Ki, M.W., Jin, S.H., Ha, J., Noh, M., 2019. A long-wave UVA filter avobenzone induces obesogenic phenotypes in normal human epidermal keratinocytes and mesenchymal stem cells. Arch. Toxicol. 93 (7), 1903–1915.

Apel, C., Joerss, H., Ebinghaus, R., 2018a. Environmental occurrence and hazard of organic UV stabilizers and UV filters in the sediment of European North and Baltic Seas. Chemosphere 212, 254–261.

Apel, C., Tang, J., Ebinghaus, R., 2018b. Environmental occurrence and distribution of organic UV stabilizers and UV filters in the sediment of Chinese Bohai and Yellow Seas. Environ. Pollut. 235, 85–94.

Balazs, A., Krifaton, C., Orosz, I., Szoboszlay, S., Kovacs, R., Csenki, Z., Urbanyi, B., Kriszt, B., 2016. Hormonal activity, cytotoxicity and developmental toxicity of UV filters. Ecotoxicol. Environ. Saf. 131, 45–53.

Balmer, M.E., Buser, H.R., Muller, M.D., Poiger, T., 2005. Occurrence of some organic UV filters in wastewater, in surface waters, and in fish from Swiss Lakes. Environ. Sci. Technol. 39 (4), 953–962.

Baron, E., Gago-Ferrero, P., Gorga, M., Rudolph, I., Mendoza, G., Zapata, A.M., Diaz-Cruz, S., Barra, R., Ocampo-Duque, W., Paez, M., Darbra, R.M., Eljarrat, E., Barcelo, D., 2013. Occurrence of hydrophobic organic pollutants (BFRs and UV-filters) in sediments from South America. Chemosphere 92 (3), 309–316.

Bens, G., 2014. Sunscreens. Adv. Exp. Med. Biol. 810, 429–463.

Bury, D., Belov, V.N., Qi, Y., Hayen, H., Volmer, D.A., Brüning, T., Koch, H.M., 2018. Determination of urinary metabolites of the emerging UV filter octocrylene by online-SPE-LC-MS/MS. Anal. Chem. 90 (1), 944–951.

Bury, D., Modick-Biermann, H., Leibold, E., Brüning, T., Koch, H.M., 2019. Urinary metabolites of the UV filter octocrylene in humans as biomarkers of exposure. Arch. Toxicol. 93 (5), 1227–1238.

Campos, D., Gravato, C., Quintaneiro, C., Golovko, O., Zlabek, V., Soares, A., Pestana, J.L.T., 2017. Toxicity of organic UV-filters to the aquatic midge Chironomus riparius. Ecotoxicol. Environ. Saf. 143, 210–216.

CEHOS, 2013. Assessment of the Endocrine Disrupting Potential of 23 UV-filters. Danish Centre on Endocrine Disrupters.

Chatelain, E., Gabard, B., Surber, C., 2003. Skin penetration and sun protection factor of five UV filters: effect of the vehicle. Skin Pharmacol. Appl. Ski. Physiol. 16 (1), 28–35.

Destatis, 2018. Press Release No. 19. Federal Statistical Office, Wiesbaden, Germany.

Diffey, B.L., 2001. When should sunscreen be reapplied? J. Am. Acad. Dermatol. 45 (6), 882–885.

DuBois, D., DuBois, E.F., 1989. A formula to estimate the approximate surface area if height and weight be known. 1916. Nutrition 5 (5), 303–311 (discussion 312-303).

Duracher, L., Blasco, L., Abdel Jaoued, A., Vian, L., Marti-Mestres, G., 2009. Irradiation of skin and contrasting effects on absorption of hydrophilic and lipophilic compounds. Photochem. Photobiol. 85 (6), 1459–1467.

Emilson, A., Lindberg, M., Forslind, B., 1993. The temperature effect on in vitro penetration of sodium lauryl sulfate and nickel chloride through human skin. Acta Derm. Venereol. 73 (3), 203–207.

European Chemical Agency ECHA Disseminated Dossier CAS 6197-30-4 (Octocrilene). https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14858/1, Accessed date: 16 July 2019.

Fitzpatrick, T.B., 1988. The validity and practicality of sun-reactive skin types I through VI. Arch. Dermatol. 124 (6), 869–871.

Forni Ogna, V., Ogna, A., Vuistiner, P., Pruijm, M., Ponte, B., Ackermann, D., Gabutti, L., Vakilzadeh, N., Mohaupt, M., Martin, P.Y., Guessous, I., Pechere-Bertschi, A., Paccaud, F., Bochud, M., Burnier, M., 2015. New anthropometry-based age- and sex-specific reference values for urinary 24-hour creatinine excretion based on the adult Swiss population. BMC Med. 13, 40.

Freitas, J.V., Lopes, N.P., Gaspar, L.R., 2015. Photostability evaluation of five UV-filters, trans-resveratrol and beta-carotene in sunscreens. Eur. J. Pharm. Sci. 78, 79–89.

Gago-Ferrero, P., Diaz-Cruz, M.S., Barcelo, D., 2012. An overview of UV-absorbing compounds (organic UV filters) in aquatic biota. Anal. Bioanal. Chem. 404 (9), 2597–2610.

Gago-Ferrero, P., Alonso, M.B., Bertozzi, C.P., Marigo, J., Barbosa, L., Cremer, M., Secchi, E.R., Domit, C., Azevedo, A., Lailson-Brito Jr., J., Torres, J.P., Malm, O., Eljarrat, E., Diaz-Cruz, M.S., Barcelo, D., 2013. First determination of UV filters in marine mammals. Octocrylene levels in Franciscana dolphins. Environ. Sci. Technol. 47 (11), 5619–5625.

Gilbert, E., Pirot, F., Bertholle, V., Roussel, L., Falson, F., Padois, K., 2013. Commonly used UV filter toxicity on biological functions: review of last decade studies. Int. J. Cosmet. Sci. 35 (3), 208–219.

Giraldo, A., Montes, R., Rodil, R., Quintana, J.B., Vidal-Linan, L., Beiras, R., 2017. Ecotoxicological evaluation of the UV filters ethylhexyl dimethyl p-aminobenzoic acid and octocrylene using marine organisms Isochrysis galbana, Mytilus galloprovincialis and Paracentrotus lividus. Arch. Environ. Contam. Toxicol. 72 (4), 606–611.

Göen, T., Helden, F. von, Eckert, E., Knecht, U., Drexler, H., Walter, D., 2016. Metabolism and toxicokinetics of 1,4-dioxane in humans after inhalational exposure at rest and under physical stress. Arch. Toxicol. 90 (6), 1315–1324.

de Groot, A.C., Roberts, D.W., 2014. Contact and photocontact allergy to octocrylene: a review. Contact Dermatitis 70 (4), 193–204.

Hexsel, C.L., Bangert, S.D., Hebert, A.A., Lim, H.W., 2008. Current sunscreen issues: 2007 Food and Drug Administration sunscreen labelling recommendations and combination sunscreen/insect repellent products. J. Am. Acad. Dermatol. 59 (2), 316–323.

Hiller, J., Klotz, K., Meyer, S., Uter, W., Hof, K., Greiner, A., Göen, T., Drexler, H., 2019. Toxicokinetics of urinary 2-ethylhexyl salicylate and its metabolite 2-ethyl-hydroxyhexyl salicylate in humans after simulating real-life dermal sunscreen exposure. Arch. Toxicol. https://doi.org/10.1007/s00204-019-02537-z. In press.

Iannuccelli, V., Coppi, G., Sergi, S., Mezzena, M., Scalia, S., 2008. In vivo and in vitro skin permeation of butyl methoxydibenzoylmethane from lipospheres. Skin Pharmacol. Physiol. 21 (1), 30–38.

ISO, 2010. 24444:2010 Cosmetics – Sun Protection Test Methods – In Vivo Determination of the Sun Protection Factor (SPF). International Organization for Standardization, Geneva, Switzerland.

Jiang, R., Roberts, M.S., Collins, D.M., Benson, H.A., 1999. Absorption of sunscreens across human skin: an evaluation of commercial products for children and adults. Br. J. Clin. Pharmacol. 48 (4), 635–637.
Kerr, A.C., 2011. A survey of the availability of sunscreen filters in the UK. Clin. Exp. Dermatol. 36 (5), 541–543.
Khemiri, R., Cote, J., Fetoui, H., Bouchard, M., 2018. Kinetic time courses of lambda-cyhalothrin metabolites after dermal application of Matador EC 120 in volunteers. Toxicol. Lett. 296, 132–138.
Klemsdal, T.O., Gjesdal, K., Bredesen, J.E., 1992. Heating and cooling of the nitroglycerin patch application area modify the plasma level of nitroglycerin. Eur. J. Clin. Pharmacol. 43 (6), 625–628.
Klotz, K., Hof, K., Hiller, J., Göen, T., Drexler, H., 2019. Quantification of prominent organic UV filters and their metabolites in human urine and plasma samples. J. Chromatogr. B 1125, 121706. https://doi.org/10.1016/j.jchromb.2019.06.033.
Krause, M., Klit, A., Blomberg Jensen, M., Soeborg, T., Frederiksen, H., Schlumpf, M., Lichtensteiger, W., Skakkebaek, N.E., Drzewiecki, K.T., 2012. Sunscreens: are they beneficial for health? An overview of endocrine disrupting properties of UV-filters. Int. J. Androl. 35 (3), 424–436.
Kunz, P.Y., Fent, K., 2006. Multiple hormonal activities of UV filters and comparison of in vivo and in vitro estrogenic activity of ethyl-4-aminobenzoate in fish. Aquat. Toxicol. 79 (4), 305–324.
Larsen, K., 1972. Creatinine assay by a reaction-kinetic principle. Clin. Chim. Acta 41, 209–217.
Li, A.J., Law, J.C., Chow, C.H., Huang, Y., Li, K., Leung, K.S., 2018. Joint effects of multiple UV filters on zebrafish embryo development. Environ. Sci. Technol. 52 (16), 9460–9467.
Mancuso, J.B., Maruthi, R., Wang, S.Q., Lim, H.W., 2017. Sunscreens: an update. Am. J. Clin. Dermatol. 18 (5), 643–650.
Manova, E., Goetz, N. von, Hauri, U., Bogdal, C., Hungerbuhler, K., 2013. Organic UV filters in personal care products in Switzerland: a survey of occurrence and concentrations. Int. J. Hyg. Environ. Health 216 (4), 508–514.
Matta, M.K., Zusterzeel, R., Pilli, N.R., Patel, V., Volpe, D.A., Florian, J., Oh, L., Bashaw, E., Zineh, I., Sanabria, C., Kemp, S., Godfrey, A., Adah, S., Coelho, S., Wang, J., Furlong, L.A., Ganley, C., Michele, T., Strauss, D.G., 2019. Effect of sunscreen application under maximal use conditions on plasma concentration of sunscreen active ingredients: a randomized clinical trial. Jama 321 (21), 2082–2091. https://doi.org/10.1001/jama.2019.5586.
Montenegro, L., Puglisi, G., 2013. Evaluation of sunscreen safety by in vitro skin permeation studies: effects of vehicle composition. Pharmazie 68 (1), 34–40.
Montenegro, L., Carbone, C., Paolino, D., Drago, R., Stancampiano, A.H., Puglisi, G., 2008. In vitro skin permeation of sunscreen agents from O/W emulsions. Int. J. Cosmet. Sci. 30 (1), 57–65.
Nash, J.F., Tanner, P.R., 2014. Relevance of UV filter/sunscreen product photostability to human safety. Photodermatol. Photoimmunol. Photomed. 30 (2–3), 88–95.
Ozaez, I., Aquilino, M., Morcillo, G., Martinez-Guitarte, J.L., 2016a. UV filters induce transcriptional changes of different hormonal receptors in Chironomus riparius embryos and larvae. Environ. Pollut. 214, 239–247.
Ozaez, I., Morcillo, G., Martinez-Guitarte, J.L., 2016b. Ultraviolet filters differentially impact the expression of key endocrine and stress genes in embryos and larvae of Chironomus riparius. Sci. Total Environ. 557–558, 240–247.
Petersen, B., Wulf, H.C., 2014. Application of sunscreen—theory and reality. Photodermatol. Photoimmunol. Photomed. 30 (2–3), 96–101.
Pinheiro, J.C., Bates, D.M., 2000. Mixed-effects Models in S and S-Plus. Springer-Verlag, New York.
Potard, G., Laugel, C., Schaefer, H., Marty, J.P., 2000. The stripping technique: in vitro absorption and penetration of five UV filters on excised fresh human skin. Skin Pharmacol. Appl. Ski. Physiol. 13 (6), 336–344.
Rom, W. N., S. Markowitz (2007). Environmental and Occupational Medicine. Philadelphia.
Ruszkiewicz, J.A., Pinkas, A., Ferrer, B., Peres, T.V., Tsatsakis, A., Aschner, M., 2017. Neurotoxic effect of active ingredients in sunscreen products, a contemporary review. Toxicol. Rep. 4, 245–259.
Scalia, S., Coppi, G., Iannuccelli, V., 2011. Microencapsulation of a cyclodextrin complex of the UV filter, butyl methoxydibenzoylmethane: in vivo skin penetration studies. J. Pharm. Biomed. Anal. 54 (2), 345–350.
Schlumpf, M., Cotton, B., Conscience, M., Haller, V., Steinmann, B., Lichtensteiger, W., 2001. In vitro and in vivo estrogenicity of UV screens. Environ. Health Perspect. 109 (3), 239–244.
Schreurs, R.H., Sonneveld, E., Jansen, J.H., Seinen, W., van der Burg, B., 2005. Interaction of polycyclic musks and UV filters with the estrogen receptor (ER), androgen receptor (AR), and progesterone receptor (PR) in reporter gene bioassays. Toxicol. Sci. 83 (2), 264–272.
Seite, S., Del Marmol, V., Moyal, D., Friedman, A.J., 2017. Public primary and secondary skin cancer prevention, perceptions and knowledge: an international cross-sectional survey. J. Eur. Acad. Dermatol. Venereol. 31 (5), 815–820.
Silva, E.S.D., Tavares, R., Paulitsch, F.D.S., Zhang, L., 2018. Use of sunscreen and risk of melanoma and non-melanoma skin cancer: a systematic review and meta-analysis. Eur. J. Dermatol. 28 (2), 186–201.
Stoeckelhuber, M., Krnac, D., Pluym, N., Scherer, M., Peschel, O., Leibold, E., Scherer, G., 2018. Human metabolism and excretion kinetics of the fragrance 7-hydroxycitronellal after a single oral or dermal dosage. Int. J. Hyg. Environ. Health 221 (2), 239–245.
Uter, W., Goncalo, M., Yazar, K., Kratz, E.M., Mildau, G., Liden, C., 2014. Coupled exposure to ingredients of cosmetic products: III. Ultraviolet filters. Contact Dermatitis 71 (3), 162–169.
Vidal-Linan, L., Villaverde-de-Saa, E., Rodil, R., Quintana, J.B., Beiras, R., 2018. Bioaccumulation of UV filters in Mytilus galloprovincialis mussel. Chemosphere 190, 267–271.
Wang, J., Pan, L., Wu, S., Lu, L., Xu, Y., Zhu, Y., Guo, M., Zhuang, S., 2016. Recent advances on endocrine disrupting effects of UV filters. Int. J. Environ. Res. Public Health 13 (8).
WHO, 1996. Biological Monitoring of Chemical Exposure in the Workplace. World Health Organization, Genf.
Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer Verlag, New York.
Witorsch, R.J., Thomas, J.A., 2010. Personal care products and endocrine disruption: a critical review of the literature. Crit. Rev. Toxicol. 40 (Suppl. 3), 1–30.
Young, A.R., Claveau, J., Rossi, A.B., 2017. Ultraviolet radiation and the skin: photobiology and sunscreen photoprotection. J. Am. Acad. Dermatol. 76 (3S1), S100–S109.
Zhang, Q.Y., Ma, X.Y., Wang, X.C., Ngo, H.H., 2016. Assessment of multiple hormone activities of a UV-filter (octocrylene) in zebrafish (Danio rerio). Chemosphere 159, 433–441.