Exposure to ZnO/TiO2 Nanoparticles Affects Health Outcomes in Cosmetics Salesclerks

Ching-Chang Lee
National Cheng Kung University
Yi-Hsin Lin
National Cheng Kung University
Wen-Che Hou
National Cheng Kung University
Meng-Han Li
National Cheng Kung University
Jung-Wei Chang (jungwei723@gmail.com)
National Yang-Ming University https://orcid.org/0000-0002-3791-5349

Research

Keywords: Nanocosmetics, Zinc oxide, Titanium dioxide, Oxidative stress markers

Posted Date: July 22nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-44825/v1

Version of Record: A version of this preprint was published at International Journal of Environmental Research and Public Health on August 21st, 2020. See the published version at https://doi.org/10.3390/ijerph17176088.

Abstract

Background: Concerns about the effects of nanoparticles (NPs) on human health are being raised by researchers because the risks of nanocosmetics like sunscreen are unknown.

Methods: We explored the association between urinary oxidative stress markers and exposure of cosmetics salesclerks to 20 cosmetics which might contain titanium dioxide (TiO_2)/zinc oxide (ZnO) NPs. We then recruited 40 cosmetics salesclerks and 24 clothing salesclerks and categorized them based on their exposure to ZnO and TiO_2 NPs.

Results: Nineteen and fifteen samples met the EU definition for TiO_2 and ZnO nanomaterials, respectively. Participants with a higher co-exposure index of ZnO and TiO_2 NPs had a significantly higher base level of urinary 8-hydroxy-2'-deoxyguanosin (8-OHdG) concentrations than the lower co-exposure group (5.82 vs. 2.85 ng/mL, $p < 0.001$). After potential confounding factors had been adjusted for, the TiO_2 and ZnO NP co-exposure index was significantly positively associated with the urinary 8-OHdG base concentration (β = 0.308, 95% CI = 0.106 to 0.510) and the creatinine-adjusted concentration (β = 0.486, 95% CI = 0.017 to 0.954).

Conclusion: Current evidence suggests that the likelihood of harm from using sunscreens containing nanoparticles might result in higher urinary 8-OHdG. However, our limited number and types of sample cosmetics might underestimate the risk.

Introduction

Recently, consumer products such as cosmetics, clothes, packaging, toys, and even food products created using nanotechnology have been gradually incorporated into our daily life, and the list is growing fast [1]. The most common cosmetic that uses nanotechnology (nanocosmetic) is sunscreen. However, studies [2, 3] have raised health concerns about nanoparticles (NPs) because their health risks are unknown. Because they are small, nanoparticles enable the skin to easily absorb nanocosmetics; thus, nanomaterials are widely used in skin care products and cosmetics. Most nanocosmetics contain titanium dioxide (TiO_2) and zinc oxide (ZnO) NPs about 50–100 nm, which are excellent for ultraviolet (UV) light shielding and transparency than are larger particles.

Concerns relating to the use of nanoparticles are two-fold. First, NPs in nanocosmetics produce more free radicals when exposed to UV light. Although healthy or psoriatic skin absorbs less TiO_2 or ZnO [4], in real-life situations, uptake through skin cracks, minor injuries, and flexed skin remains to be evaluated. In addition, TiO_2 NPs sometimes penetrate the skin and might become a major concern for people with healthy skin. Many studies [5–7] have reported that exposure to TiO_2 or ZnO NPs might cause cell death and allergic reactions, and it might significantly raise urinary 8-hydroxy-2'-deoxyguanosin (8-OHdG) levels and concentrations of inflammatory marker like IL-6, IL-8, and TNF-α.

Moreover, TiO_2 and ZnO NPs absorb significant UV radiation, which, in aqueous media, produces hydroxyl species. These species might substantially damage DNA [8, 9], therefore raising health concerns about sunscreens [10].

According to one commercial survey [11], the nano-ingredients of nanocosmetics are unclearly labeled because of the strict regulations and complicated certification system. Thus, consumers are liable to expose themselves to NPs without realizing it. Critical information such as nanoparticle size and concentrations are not disclosed for most consumer products; therefore, the actual health hazard of these products remain largely unknown.

Cosmetics salesclerks work for retail stores or cosmetics distributors. Because they recommend cosmetics to customers, they often apply nanocosmetics on their own skin. Information about the potential risks of by using products containing NPs is scarce; thus, we explored the association between urinary oxidative stress markers and exposure to TiO_2 and ZnO NPs in nanocosmetics salesclerks.

Materials And Methods

Sunscreen selection and analysis

To understand the types and number of consumer products available on the market that have NPs, we used the Nanotechnology Consumer Products Inventory, created by the Woodrow Wilson International Center for Scholars and the Project on Emerging Nanotechnologies, and market research studies, and made sure that the products were available in Taiwan [12]. We then purchased cosmetics which we thought would contain TiO_2 or ZnO NPs, and we used single-particle inductively coupled plasma-mass spectrometry (sp-ICP-MS) to analyze the content, concentration, and size of the NPs.

Sample preparation for sp-ICP-MS analysis

Ionic Ti and Zn ICP-MS standards, TiO_2 (P_{25}) and ZnO nanopowder (< 100 nm/particle), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nitric acid (HNO_3) (67-70% [w/w], Ultrex II) was purchased from J. T. Baker (Phillipsburg, NJ, USA). Ninety-seven percent [w/w] sulphuric acid (H_2SO_4) and 30% [w/w] hydrogen peroxide (H_2O_2) were purchased from Honeywell (Minato City, Japan). Citrate-coated 50-nm gold nanoparticles (AuNPs) were purchased from nanoComposix Inc. (San Diego, CA, USA). All other chemicals were purchased from Sigma-Aldrich or J. T. Baker. All aqueous samples were prepared with water purified by a Millipore Synergy ultrapure water system (≥ 18.0 MΩ) (Merck Ltd., Taipei, Taiwan). After all the samples had been homogenized, 0.2 g or more of the sunscreen sample was dispersed in a 1% Triton X-100 aqueous solution to make a 0.1% (v/v) suspension. Here, an aliquot of 50-mg product lotion was then added to a 1% (v/v) Triton X-100 aqueous solution (50 mL in centrifuge tubes), resulting in a 0.1% (w/v) suspension. The mixture was sonicated and vortexed until no aggregates could be seen.

sp-ICP-MS method

The typical instrument condition and settings used sp-ICP-MS analysis are given in Table S1. Aqueous samples were nebulized using a concentric nebulizer in a cyclone spray chamber and ionized using argon plasma. The sample flow rate (range: 0.28-0.36 mL/min) was determined daily. Particle size quantification was based on a calibration curve constructed from a 2% HNO_3 blank and five concentration levels of dissolved Ti and Zn standard solutions (range: 0.1 to 10 μg/L) [13]. To measure transport efficiency (η), 50 nm standard gold nanoparticles (AuNPs) (at ~10^5 particles/mL) was spiked into the samples as described by Pace et al. The transport efficiencies were in the range of 4.5-7.0% during this study.

The signal intensity (INP) obtained from sample analysis was converted to the diameter by estimating the size of the spherical TiO_2 particle using Eq. (1):

$$d = \left(\frac{6 \times I_{NP}}{R \times f_a \times \rho \times \pi}\right)^{1/3} \qquad \text{(EQ 1)}$$

where d is the diameter; *R*, which defines the detector sensitivity of the instrument, is determined from the ionic standards; f_a is the mass fraction of the metal element in the chemical formula of an NP; and ρ is the mass density. Size distribution can then be obtained by plotting *d* against the frequency of NP occurrence. NP concentration (N_p) was determined using Eq. (2):

$$N_p = \frac{f_p}{q \times t_s \times \eta} \qquad \text{(EQ 2)}$$

where f_p is the number of particles resolved per analysis; *q* is the sample flow rate; t_s is the scan time per analysis; and η is the transport efficiency. The sp-ICP-MS method used in this study can detect NPs in the concentration range of 1,000-100,000 particles/mL with quantitative recovery and good sizing capability in complex matrices such as wastewater, based on our previous results (not shown).

For each sample, the isotopes ^{47}Ti and ^{64}Zn were detected using 0.1 ms dwell time for a total measurement time of 60 s (= 600,000 data points).

After each run, software (Syngistix Nano Application Module 1; PerkinElmer, Taipei, Taiwan) automatically integrated the peak area of each single particle and generated information about particle size distribution, particle concentration, and dissolved concentration.

Recruiting participants

We recruited full-time cosmetics salesclerks in retail stores or cosmetics product distributors in department stores for the high-exposure group and clothing salesclerks in the same department stores in southern Taiwan for the control group. We first excluded recruits who had immune dysfunction or who frequently used general nanotechnology-based consumer products. We also excluded exposure group recruits who did not demonstrate products for customers. The Human Ethics Committee of National Cheng Kung University Hospital approved the study protocol (#: B-ER-105-416). All participants provided written informed consent. Finally, the exposure group contained 40 salesclerks and the control group contained 24.

Sample collection

All glassware was washed with organic solvents and packed with aluminum foil before we collected samples. From January 2 to May 8, 2017, urine samples were collected from each participant on four separate occasions. On each occasion, we collected two urine samples (20-30 mL), one pre-shift (first morning spot urine) on Thursday and another post-shift on Sunday. We conducted interviews to determine exposure scenarios and filled in standardized nanomaterials exposure questionnaires to obtain demographic characteristics.

Daily exposure dose and cumulative risk calculation

In the present study, we use an exposure index, which integrated the nanoparticle concentration of the cosmetics and the usage of nano-consumer products to evaluate the relationship between oxidative stress and chronic TiO_2 or ZnO NP exposure, which is shown as Eq. (3):

$$\text{Exposure Index (particles kg}^{-1}\text{ day}^{-1}\text{)} = \frac{C \times \text{Exp. D}}{BW} \times \frac{W\_hr}{24} \times \frac{W\_day}{365} \times \frac{ED}{AT} \qquad \text{(EQ 3)}$$

where C (particles/g) is the concentration of sunscreen; Exp. D is the exposure dose, which was calculated from the frequency of using sunscreen and the number of salesclerks; Working hours (W_hr), working days (W_day), seniority (ED), and body weight (BW) were collected from the questionnaire. Life span (AT) was taken from exposure-factor surveys [14].

Determining8-hydroxy-2´-deoxyguanosine (8-OHdG)

A competitive enzyme-linked immunosorbent assay (ELISA) (BIOXYTECH® 8-OHdG-EIA™ kit; OXIS Health Products Inc., Portland, OR, USA) was used to quantitatively measure oxidative DNA damage in urine samples. Briefly, after the necessary treatment, cellular DNA was isolated using a DNA extraction kit (iNtRON Biotechnology Inc., Sungnam, South Korea). The quantity of 8-OHdG, a deoxyriboside form of 8-oxoguanine in the DNA, was determined using a microplate reader on a standard curve measured at 450 nm absorbance.

Statistical analysis

We used descriptive statistics to describe the distributions of NP demographic data and NP size of TiO_2 and ZnO.

The participants were categorized into two groups based on their professions. The Kruskal-Wallis test was used to assess differences in oxidative stress levels and demographic characteristics between the two groups. We used a χ^2 test to determine the differences in demographic characteristics between the two groups. We also used a multivariate linear regression analysis to evaluate the association between oxidative stress levels and exposure to TiO_2 and ZnO.

SPSS 22 (IBM Corp., Armonk, NY, USA) was used for all statistical analyses. Significance was set at $p < 0.05$.

Results

Size and concentration of TiO_2 and ZnO NPs in sunscreens

After we completed our market survey of cosmetic products containing ZnO or TiO_2, we bought 20 products (all milky or cream cosmetics with UV protection) to analyze the NPs. The SP ICP-MS geometric mean diameter (nm), particle number concentration (particles/g), and metal oxide content (% weight) are reported in Table 1. TiO_2 NPs were detected in 19 sunscreens, with geometric mean diameter (Dg) values from 71.4 to 112 nm; ZnO NPs were detected in all sunscreens, with Dg values from 57.7 to 144.4 nm. The TiO_2 weight content in sunscreens ranged from 1 to 30.7% and that of ZnO from 1 to 9.14%. Many ZnO NPs were detected in six sunscreens (#002, #005, #007, #011, #013, and #020) with Dg values from 98.2 to 144.4 nm; few ZnO NPs (9.14% at maximum) were detected in all sunscreens (Table 2).

Table 1
Size, number and weight concentration of TiO_2 in sunscreens by SP-ICP-MS.

Sample ID	SPF	Geo-mean size (nm)	TiO_2 # concentration (#/g product)	Mode size (nm)	Fraction of nanosized TiO_2[1] (%)	TiO_2 weight percentage[2] (%)	TiO_2 labeling
#001	50+	98.8	6.04×10^{9}	55.6	64	0.01	No
#002	30	85.2	1.13×10^{13}	62.3	77	3.17	Yes
#003	40	75.1	2.00×10^{13}	60.3	91	2.40	Yes
#004	0	268.3	4.91×10^{12}	111.0	0	30.07	Yes
#005	50+	91.8	1.50×10^{12}	61.3	66	0.62	Yes
#006	15	72.8	1.02×10^{12}	53.7	86	0.16	Yes (1.23%)
#007	50+	71.8	1.25×10^{11}	52.7	84	0.03	No
#008	50	74.6	7.86×10^{11}	58.0	85	0.10	Yes
#009	50	75.6	1.06×10^{11}	52.8	80	0.03	Yes
#010	19	71.4	2.23×10^{12}	54.0	89	0.55	Yes
#011	50+	86.9	2.15×10^{11}	65.6	78	0.06	Yes
#012	50	81.8	7.00×10^{12}	66.0	85	1.23	Yes
#013	50+	101.5	1.83×10^{7}	66.9	65	0.00	No
#014	50	79.6	7.34×10^{12}	63.2	85	1.23	Yes
#015	15	107.8	2.54×10^{12}	63.7	57	2.84	Yes
#016	50	94.1	8.39×10^{12}	56.6	61	3.06	Yes
#017	24	82.2	1.76×10^{12}	62.6	80	0.37	Yes
#018	50+	106.4	3.60×10^{10}	64.1	54	0.03	No
#019	20	74.3	5.09×10^{12}	56.1	87	1.21	Yes
#020	25	112.0	1.14×10^{12}	68.0	50	1.24	Yes

Table 2
Size, number and weight concentration of ZnO in sunscreens by SP-ICP-MS.

Sample ID	**SPF**	**Geo-mean size (nm)**	**ZnO # concentration (#/g product)**	**Mode size (nm)**	**Fraction of nanosized ZnO[1] (%)**	**ZnO weight percentage[2] (%)**	**ZnO labelled labeling**
#001	50+	57.7	3.66×10^{7}	51.6	95	0.00	No
#002	30	102.3	4.51×10^{11}	83.0	53	0.22	No
#003	40	66.2	2.22×10^{7}	67.1	94	0.00	No
#004	0	61.7	1.64×10^{8}	62.4	100	0.00	No
#005	50+	98.2	2.36×10^{12}	72.1	30	1.72	Yes (12.53%)
#006	15	69.7	4.29×10^{7}	59.4	88	0.00	No
#007	50+	136.0	9.19×10^{12}	91.5	21	9.14	Yes (9.45%)
#008	50	55.6	1.72×10^{8}	48.5	98	0.00	No
#009	50	67.0	1.37×10^{7}	50.2	89	0.00	No
#010	19	65.2	5.42×10^{7}	55.3	93	0.00	No
#011	50+	101.0	6.16×10^{11}	96.1	39	0.37	Yes (7.36%)
#012	50	99.1	1.27×10^{7}	80.5	60	0.00	No
#013	50+	144.4	6.98×10^{11}	103.3	16	1.22	Yes (9.59%)
#014	50	92.2	1.68×10^{8}	59.0	69	0.00	No
#015	15	67.7	1.51×10^{9}	57.3	97	0.00	No
#016	50	88.7	1.51×10^{8}	73.4	79	0.00	No
#017	24	96.0	1.48×10^{8}	78.9	74	0.00	No
#018	50+	91.6	3.02×10^{6}	60.2	62	0.00	No
#019	20	77.7	1.32×10^{8}	72.4	86	0.00	No
#020	25	106.3	4.34×10^{12}	76.4	42	2.47	No

Although the analysis results showed that all the products contained ZnO and TiO_2, ZnO and TiO_2 were not labeled as ingredients on some products. The measurement of TiO_2 indicates that 19 samples met the EU definition for nanomaterials (> 50% by number of particles with a size < 100 nm), and the mode sizes ranged from 52 to 68 nm. In another analysis, 15 products met the EU definition for nanomaterials in ZnO: mode sizes ranged from 48 to 83 nm.

Study population

We recruited 40 department store cosmetics salesclerks and 24 clothing salesclerks. The average age of clothing salesclerks was higher than that of cosmetics salesclerks (42.2 vs. 27.3 years, $p < 0.001$) (Table 3). Moreover, the clothing salesclerks had a significantly higher average BMI than cosmetics salesclerks did (23.2 vs. 20.7 kg/m^2, $p < 0.001$).

Table 3
Demographic characteristics of study population grouped by sampling site

	Cosmetics salesclerks (n = 40)	Clothing salesclerks (n = 24)	$p^{a,b}$
Age (years)[c]	27.3 (20–47)	42.2 (23–54)	< 0.001
Weight (kg)[d]	54.4 (6.56)	60.6 (10.1)	0.004
Height (cm)[d]	161.8 (5.27)	161.5 (6.62)	0.812
BMI	20.7 (2.0)	23.2 (3.28)	< 0.001
Marital status[e]			
Unmarried/Divorced	29 (72.5)	11 (45.9)	0.033
Married	11 (27.5)	13 (54.2)	
Educational level			
≤ High school	15 (37.5)	17 (70.9)	0.069
Tech school	4 (10.0)	2 (8.3)	
Bachelor’s degree	21 (52.5)	5 (20.8)	
Monthly income (NT$)			
< 24999	11 (27.5)	9 (37.5)	0.048
25000 ~ 29999	5 (12.5)	9 (37.5)	
30000 ~ 34999	15 (37.5)	5 (20.8)	
35000 ~ 39999	5 (12.5)	1 (4.2)	
40000 ~ 44999	4 (10.0)	0 (0.0)	
Seniority (years)	4.72 (5.70)	11.9 (7.70)	< 0.001
Working time (day/month)	20.5 (2.80)	21.3 (2.63)	0.262

[a] Continuous variables between two groups were compared using the Kruskal-Wallis test.

[b]Categorical variables were compared with χ^2 test.

[c]Expressed as Mean (range); [d]Expressed as Mean ± SD; [e]n (%).

[f]Once a week at least *$p < 0.05$; **$p < 0.01$.

	Cosmetics salesclerks (n = 40)	Clothing salesclerks (n = 24)	$p^{a,b}$
Weekday working time (h/day)	8.59 (0.53)	7.96 (2.13)	0.078
Weekend working time (h/day)	8.70 (0.71)	8.52 (2.03)	0.611
Exposed to Tobacco Smoke Exposure			
Active	7 (17.5)	2 (8.3)	0.264
Secondhand	13 (32.5)	10 (41.7)	0.317
Alcohol drinker[f]	5 (12.5)	0 (0.0)	0.086
Tea drinker[f]	26 (65.0)	13 (54.2)	0.275
Coffee drinker[f]	15 (37.5)	14 (58.3)	0.087

[a] Continuous variables between two groups were compared using the Kruskal-Wallis test.

[b]Categorical variables were compared with χ^2 test.

[c]Expressed as Mean (range); [d]Expressed as Mean ± SD; [e]n (%).

[f]Once a week at least *$p < 0.05$; **$p < 0.01$.

Moreover, the cosmetics salesclerks had higher education and economic levels than clothing salesclerks did. Cosmetics salesclerks had more weekday working time than clothing salesclerks did.

Cosmetics salesclerks used more cosmetics (lotions, lipstick, powder foundation, and eye shadow) than clothing salesclerks did (Table S2).

Urinary 8-OHdG analysis in nanocosmetics salesclerks

All salesclerks were first categorized based on their job title. Baseline 8-OHdG concentrations in the cosmetics salesclerks were significantly higher than those in the clothing salesclerks (5.42 vs. 2.53 ng/mL, $p = 0.001$) in Thursday pre-shift urine, but there were no significant differences between the Sunday post-shift concentrations, not even after creatinine had been adjusted for. We excluded salesclerks with abnormal creatinine (< 30 or > 300 mg/dL) levels. Baseline 8-OHdG concentrations in the cosmetics salesclerks were significantly higher than in the clothing salesclerks in both Thursday pre-shift and Sunday post-shift urine (5.41 vs. 2.85 ng/mL, $p = 0.004$; 4.90 vs. 2.85 ng/mL, $p = 0.037$) (Table S3).

All salesclerks were then categorized based on the co-exposure index of ZnO and TiO_2 NPs (Table 4). Baseline 8-OHdG concentrations in the high-exposure group were significantly higher than in the low-exposure group (5.82 and 2.85 ng/mL, $p = 0.001$).

Table 4
Oxidative stress analytical results grouped by Co-Exposure index integrating ZnO and TiO_2 NPs

	Thursday Pre-shift Urine			**Sunday Post-shift Urine**		
	High (n = 32)	**Low (n = 32)**	**P-value[a,b]**	**High (n = 32)**	**Low (n = 32)**	**P-value**
8-OHdG (ng/mL)[c]	5.82 (3.34)	2.85 (2.91)	< 0.001**	4.44 (3.87)	2.91 (3.14)	0.086#
8-OHdG (μg/g creatinine)[c]	6.89 (13.0)	3.22 (2.36)	0.122	4.94 (6.01)	3.78 (3.02)	0.332
Creatinine (mg/dL) [c]	144 (105)	84.2 (75.7)	0.011*	102 (68.1)	75.5 (47.7)	0.072#
Protein [d]	17 (53.1)	8 (25.0)	0.020*	8 (25.0)	5 (15.6)	0.268
Glucose urine [d]	0 (0.0)	0 (0.0)	-	0 (0.0)	1 (3.1)	0.500
Bilirubin [d]	0 (0.0)	0 (0.0)	-	0 (0.0)	0 (0.0)	-
Urobilinogen [d]	0 (0.0)	0 (0.0)	-	0 (0.0)	0 (0.0)	-
Occult Blood [d]	5 (15.6)	4 (12.5)	0.500	3 (9.4)	4 (12.5)	0.500
8-OHdG (ng/mL) [e]	5.90 (2.72)	3.12 (2.98)	0.001**	4.84 (3.84)	3.30 (3.24)	0.112
8-OHdG (μg/g creatinine) [e]	4.41 (1.84)	3.02 (2.20)	0.016*	5.17 (6.19)	3.68 (2.84)	0.258
Creatinine (mg/dL) [e]	147 (61.6)	86.8 (55.5)	< 0.001**	114 (65.1)	85.2 (57.8)	0.064#

[a] Continuous variables between two groups were compared using Kruskal-Wallis test, [b] Categorical variables were compared with Chi-Square test, [c] Expressed as Mean (SD), [d] Abnormal n (%), [e] Exclude those who with abnormal creatinine (WHO Reference: 30–300 mg/dL), #P < 0.1, * $P < 0.05$; ** $P < 0.01$

The exposure indices of TiO_2 NPs were significantly positively associated with baseline and creatinine-adjusted 8-OHdG concentrations (β = 0.417, $p < 0.001$, and β = 0.334, $p < 0.001$) (Table S4).

After adjusting potential confounding factors, the exposure index of TiO_2 NPs were significantly positively associated with both the urinary 8-OHdG original and creatinine-adjusted concentration (β =

0.383, 95%CI = 0.176 to 0.589, and β = 0.648, 95%CI = 0.167 to 1.131) (Table 5). Moreover, after adjusting potential confounding factors, the TiO_2 and ZnO NPs co-exposure index were significantly positively associated with both the urinary 8-OHdG original and creatinine-adjusted concentration (β = 0.308, 95%CI = 0.106 to 0.510, and β = 0.486, 95%CI = 0.017 to 0.954) (Table 5).

Table 5
Multiple regression analysis of urinary 8-OHdG concentration and exposure index [a, b]

Exposure Index [c]	ZnO NPs	TiO_2 NPs	ZnO and TiO_2 NPs
Urinary 8-OHdG (ng/mL) [d]	β = -0.047 (-0.272, 0.177)	β = 0.383 ** (0.176, 0.589)	β = 0.308 ** (0.106, 0.510)
Urinary 8-OHdG (μg/g creatinine)	β = -0.087 (-0.600, 0.426)	β = 0.649 ** (0.167, 1.131)	β = 0.486 * (0.017, 0.954)

[a] Multiple regression analysis; [b] Adjusted for BMI, smoking, alcohol drinking and tea drinking habits; [c] log-transformations of exposure index was used; [d] Expressed as coefficient β and 95% CI; * $P < 0.05$, ** $P < 0.01$

Discussion

Findings

Our most important finding was that all the products we bought and analyzed contained ZnO and TiO_2; however, some of the products did not include ZnO and TiO_2 as ingredients on their labels. Nineteen TiO_2 samples met the EU definition for nanomaterials (> 50% < 100 nm by number of particles/g); mode sizes ranged from 52 to 68 nm. Another analysis showed that 15 ZnO samples met the EU definition for ZnO nanomaterials. Smijs and Pavel (2011) reported that TiO_2 was more effective in the UVB range and ZnO in the UVA range [15]. Broadband UV protection might be more significant were these two chemicals combined. We also found that ZnO and TiO_2 NPs had been measured in commercial sunscreens [16, 17].

The significantly positive correlation between urinary 8-OHdG levels and the exposure index of TiO_2 NPs and the co-exposure index integrating TiO_2 and ZnO NPs indicated that exposure to NPs might affect the oxidative stress of salesclerks despite inconsistent evidence that NPs are small enough to penetrate the epidermis and the blood stream [18].

Most sunscreens that contain will lead to the photo-oxidation of phenol and damage human DNA using TiO_2 as a catalyst under sunlight (Dunford et al., 1997). In contrast to Gulson et al., (2010), which reported more ZnO in the blood and urine of human volunteers after a five-day continuous application of ZnO NPs-containing sunscreen [19]. Moreover, James et al., 2013 claimed that a small amount of Zn (either as (NPs or free ions) can penetrate healthy human skin, enter the circulation, and stimulate an immune response [20]; they concluded that macrophages are important for protecting the body against the cytotoxic effects of ZnO NPs.

Martorano et al. (2010) reported that UVB irradiation causes Zn^{2+} dissociation in ZnO sunscreen and, therefore, the accumulation of imbalanced Zn^{2+} causes cytotoxicity and oxidative stress [21]. ZnO or TiO_2 NPs can produce a series of biochemical and cellular signaling events—ROS induction, DNA damage, and cell death mediated by activating NF-κB, a transcription factor of proinflammatory responses [22], proinflammatory cytokine secretion [23], and endoplasmic reticulum stress induction [24]. Of great importance to the potential adverse effects of NPs in sunscreens, TiO_2 and ZnO NPs has been found with cytotoxicity in different human skin models, principally HaCa T cells (immortalized human keratinocytes), human or animal-derived skin samples, and human volunteers [25]. When HaCa T cells were incubated with 10 to 500 μg mL^{-1} of TiO_2 NPs for 2 h, a significant reduction in cell viability and induction of ROS also occurred [26]. After daily oral administration of TiO_2 for 6 months, lower body weight and significant kidney pathology were found in mice, and could be possibly attributable to NP-induced oxidative stress [27]. TiO_2 NPs caused liver injury and induced oxidative stress and DNA damage in hepatocytes of mice when administered for 2 weeks at doses > 100 mg/kg [28].

Limitations

This study has limitations. High exposure levels to TiO_2 and ZnO NPs might raise 8-OHdG concentrations. We analyzed only liquid and milky products, but even TiO_2 and ZnO NPs might be added to other solid, cream, or spray-type cosmetics as well; therefore, it might underestimate the risk. Hence, more product categories must be included to conduct further simultaneous exposure assessment. The benefits of sunscreens reduce skin cancer risk, which far outweighs the potential risks of long-term use. Nano-sized TiO_2 might also be widely used in toothpastes [29, 30].

Conclusions

Current evidence suggests that the likelihood of harm from the use of sunscreens that contain NPs is low. Sunscreens reduce skin cancer risk, which far outweighs the potential risks of long-term use. Further research is required to support our findings.

Abbreviations

NPs
nanoparticles; TiO_2:titanium dioxide; ZnO:zinc oxide; 8-OHdG:8-hydroxy-2'-deoxyguanosin, UV:ultraviolet; sp-ICP-MS:single-particle inductively coupled plasma-mass spectrometry; ELISA:enzyme-linked immunosorbent assay

Declarations

Acknowledgments

We would like to thank Mrs. Yi-Hsin Lin and Mrs. Meng-Han Li for their assistance in data collection, specimen collection and method development as well as Dr. Wen-Che Hou for his assistance in sample pretreatment and conducting sp-ICP-MS analysis. We are also deeply grateful to the research collaboration of the National Cheng Kung University, Prof. Ching-Chang Lee for the support in sampling and data analysis.

Authors' Contributions

YHL and MHL conceived and designed the experiments, WCH performed the experiments; JWC contributed tools for reagents, materials, and analysis; CCL and JWC analyzed the data, and JWC wrote the paper; Specimen collection as well as sample arrangement and preparations were managed by YHL; JWC and CCL contributed to critical revision of the manuscript.

Funding

This work was supported by grants from the Ministry of Science and Technology (MOST 106-2314-B-010-057-).

Availability of data and materials

The data included in the manuscript is available from the responsible author,

Jung-Wei Chang, upon request (jungwei723@gmail.com).

Ethics approval and consent to participate

The study was approved by the Human Ethics Committee of National Cheng Kung University Hospital, Tainan, Taiwan and are in accordance with the Declaration of Helsinki. Before sampling, all participants provided written informed consent.

Consent for publication

Not applicable.

Competing interests

All authors declared that they have no conflicts of interest.

Author details

Ching-Chang Lee[a,b], cclee@mail.ncku.edu.tw

Yi-Hsin Lin[a], yihsin19940805@gmail.com

Wen-Che Hou[c], whou@mail.ncku.edu.tw

Meng-Han Li[c], amiable0330@gmail.com

Jung-Wei Chang[d], jungwei723@gmail.com

[a]Department of Environmental and Occupational Health, College of Medicine, National Cheng Kung University, Tainan, Taiwan; [b]Research Center for Environmental Trace Toxic Substances, National Cheng Kung University, Tainan, Taiwan; [c]Department of Environmental Engineering, National Cheng Kung University, Tainan, Taiwan; [d]Institute of Environmental and Occupational Health Sciences, School of Medicine, National Yang-Ming University, Taipei, Taiwan;

References

1. Thomas T, Thomas K, Sadrieh N, Savage N, Adair P, Bronaugh R. Research strategies for safety evaluation of nanomaterials, part VII: evaluating consumer exposure to nanoscale materials. Toxicol Sci 2006; 91:14-19.
2. Aro H, Dahms G. Compatibility of micro-fine titanium dioxide with organic UV filters. Cosmet Toiletries Manufacture Worldwide 2004:115-118.
3. Tyner KM, Wokovich AM, Godar DE, Doub WH, Sadrieh N. The state of nano-sized titanium dioxide (TiO_2) may affect sunscreen performance. Int J Cosmet Sci 2011;33:234-244.
4. Wiesenthal A, Hunter L, Wang S, Wickliffe J, Wilkerson M. Nanoparticles: small and mighty. Int J Dermatol 2011;50:247-254.
5. Carlson, S. M. Hussain, A. M. Schrand, L. K. Braydich-Stolle, K. L. Hess, R. L. Jones, Schlager JJ. Unique cellular interaction of silver nanoparticles_ size-dependent generation. J Phys Chem B 2008;112:13608–13619.
6. Shakeel M, Jabeen F, Shabbir S, Asghar MS, Khan MS, Chaudhry AS. Toxicity of Nano-Titanium Dioxide (TiO_2-NP) Through Various Routes of Exposure: a Review. Biol Trace Elem Res 2016;172:1-36.
7. Trouiller B, Reliene R, Westbrook A, Solaimani P, Schiestl RH. Titanium dioxide nanoparticles induce DNA damage and genetic instability in vivo in mice. Cancer research 2009;69:8784-8789.
8. Dunford R, Salinaro A, Cai L, Serpone N, Horikoshi S, Hidaka H, Knowland J. Chemical oxidation and DNA damage catalysed by inorganic sunscreen ingredients. FEBS letters 1997;418:87-90.
9. Hidaka H, Horikoshi S, Serpone N, Knowland J. In vitro photochemical damage to DNA, RNA and their bases by an inorganic sunscreen agent on exposure to UVA and UVB radiation. Journal of Photochemistry and Photobiology a-Chemistry J Photochem Photobiol, A 1997;111:205-213.
10. Oberdorster G, Oberdorster E, Oberdorster J. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 2005;113:823-839.

11. Friends of the Earth (2006) Nanomaterials, Sunscreens, and Cosmetics: Small Ingredients, Big Risks. Available at http://emergingtech.foe.org.au/wp-content/uploads/2014/06/nano-cosmetics-report-2MB.pdf, accessed on Sept. 1, 2019.
12. Vance ME, Kuiken T, Vejerano EP, McGinnis SP, Hochella MF, Jr., Rejeski D, Hull MS. Nanotechnology in the real world: Redeveloping the nanomaterial consumer products inventory. Beilstein J Nanotechnol 2015;6:1769-1780.
13. Pace HE, Rogers NJ, Jarolimek C, Coleman VA, Higgins CP, Ranville JF. Determining transport efficiency for the purpose of counting and sizing nanoparticles via single particle inductively coupled plasma mass spectrometry. Anal Chem 2011;83:9361-9369.
14. 2008. Compilation of Exposure Factors, Department of Health, Executive Yuan, Taiwan.
15. Smijs TG, Pavel S. Titanium dioxide and zinc oxide nanoparticles in sunscreens: focus on their safety and effectiveness. Nanotechnol Sci Appl 2011;4:95-112.
16. Lu PJ, Huang SC, Chen YP, Chiueh LC, Shih DY. Analysis of titanium dioxide and zinc oxide nanoparticles in cosmetics. J Food Drug Anal 2015;23:587-594.
17. Wokovich A, Tyner K, Doub W, Sadrieh N, Buhse LF. Particle size determination of sunscreens formulated with various forms of titanium dioxide. Drug Dev Ind Pharm 2009;35:1180-1189.
18. Jansen R, Osterwalder U, Wang SQ, Burnett M, Lim HW. Photoprotection: part II. Sunscreen: development, efficacy, and controversies. J Am Acad Dermatol 2013;69:867 e861-814; quiz 881-862.
19. Gulson B, McCall M, Korsch M, Gomez L, Casey P, Oytam Y, Taylor A, McCulloch M, Trotter J, Kinsley L, Greenoak G. Small amounts of zinc from zinc oxide particles in sunscreens applied outdoors are absorbed through human skin. Toxicol Sci 2010;118:140-149.
20. James SA, Feltis BN, de Jonge MD, Sridhar M, Kimpton JA, Altissimo M, Mayo S, Zheng C, Hastings A, Howard DL, Paterson DJ, Wright PF, Moorhead GF, Turney TW, Fu J. Quantification of ZnO nanoparticle uptake, distribution, and dissolution within individual human macrophages. ACS nano 2013;7:10621-10635.
21. Martorano LM, Stork CJ, Li YV. UV irradiation-induced zinc dissociation from commercial zinc oxide sunscreen and its action in human epidermal keratinocytes. J Cosmet Dermatol 2010;9:276-286.
22. Setyawati MI, Tay CY, Leong DT. Mechanistic Investigation of the Biological Effects of SiO_2, TiO_2, and ZnO Nanoparticles on Intestinal Cells. Small 2015;11:3458-3468.
23. De Angelis I, Barone F, Zijno A, Bizzarri L, Russo MT, Pozzi R, Franchini F, Giudetti G, Uboldi C, Ponti J, Rossi F, De Berardis B. Comparative study of ZnO and TiO_2 nanoparticles: physicochemical characterisation and toxicological effects on human colon carcinoma cells. Nanotoxicology 2013;7:1361-1372.
24. Yu KN, Chang SH, Park SJ, Lim J, Lee J, Yoon TJ, Kim JS, Cho MH. Titanium Dioxide Nanoparticles Induce Endoplasmic Reticulum Stress-Mediated Autophagic Cell Death via Mitochondria-Associated Endoplasmic Reticulum Membrane Disruption in Normal Lung Cells. PloS ONE 2015;10:e0131208.

25. Crosera M, Prodi A, Mauro M, Pelin M, Florio C, Bellomo F, Adami G, Apostoli P, De Palma G, Bovenzi M, Campanini M, Filon FL. Titanium Dioxide Nanoparticle Penetration into the Skin and Effects on HaCaT Cells. Int J Environ Res Public Health 2015;12:9282-9297.
26. Rancan F, Nazemi B, Rautenberg S, Ryll M, Hadam S, Gao Q, Hackbarth S, Haag SF, Graf C, Ruhl E, Blume-Peytavi U, Lademann J, Vogt A, Meinke MC. Ultraviolet radiation and nanoparticle induced intracellular free radicals generation measured in human keratinocytes by electron paramagnetic resonance spectroscopy. Skin Res Technol 2014;20:182-193.
27. Gui S, Li B, Zhao X, Sheng L, Hong J, Yu X, Sang X, Sun Q, Ze Y, Wang L, Hong F. Renal injury and Nrf2 modulation in mouse kidney following chronic exposure to TiO_2 J Agric Food Chem 2013;61:8959-8968.
28. Shukla RK, Kumar A, Vallabani NV, Pandey AK, Dhawan A. Titanium dioxide nanoparticle-induced oxidative stress triggers DNA damage and hepatic injury in mice. Nanomedicine (Lond). 2014;9:1423-1434.
29. Sobek A, Bejgarn S, Ruden C, Molander L, Breitholtz M. In the shadow of the Cosmetic Directive--inconsistencies in EU environmental hazard classification requirements for UV-filters. Sci Total Environ 2013;461-462:706-711.
30. Weir A, Westerhoff P, Fabricius L, Hristovski K, von Goetz N. Titanium dioxide nanoparticles in food and personal care products. Environ Sci Technol 2012;46:2242-2250.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ResearchHighlights2019526.doc
- TableofContentsArt.docx
- SupplementaryTable.docx