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REVIEW OF TITANIUM DIOXIDE NANOPARTICLE PHOTOTOXICITY: DEVELOPING A PHOTOTOXICITY RATIO TO CORRECT THE ENDPOINT VALUES OF TOXICITY TESTS

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Abstract: Titanium dioxide nanoparticles are photoactive and produce reactive oxygen species under natural sunlight. Reactive oxygen species can be detrimental to many organisms, causing oxidative damage, cell injury, and death. Most studies investigating TiO_2 nanoparticle toxicity did not consider photoactivation and performed tests either in dark conditions or under artificial lighting that did not simulate natural irradiation. The present study summarizes the literature and derives a phototoxicity ratio between the results of nano-titanium dioxide (nano-TiO_2) experiments conducted in the absence of sunlight and those conducted under solar or simulated solar radiation (SSR) for aquatic species. Therefore, the phototoxicity ratio can be used to correct endpoints of the toxicity tests with nano-TiO_2 that were performed in absence of sunlight. Such corrections also may be important for regulators and risk assessors when reviewing previously published data. A significant difference was observed between the phototoxicity ratios of 2 distinct groups: aquatic species belonging to order Cladocera, and all other aquatic species. Order Cladocera appeared very sensitive and prone to nano-TiO_2 phototoxicity. On average nano-TiO_2 was 20 times more toxic to non-Cladocera and 1867 times more toxic to Cladocera (median values 3.3 and 24.7, respectively) after illumination. Both median value and 75% quartile of the phototoxicity ratio are chosen as the most practical values for the correction of endpoints of nano-TiO_2 toxicity tests that were performed in dark conditions, or in the absence of sunlight. *Environ Toxicol Chem* 2015;34:1070–1077.

Keywords: Titanium dioxide Nanoparticles Photoxicity Simulated solar radiation Photoactivation

INTRODUCTION

Titanium dioxide (TiO_2) is a component of many sunscreens, soaps, shampoos, toothpastes, cosmetics, paper products, plastics, ink, paint, and building materials [1] in both its bulk form and its nanoform. It is also used in human food as a colorant and inactive ingredient, where it can also be present in both forms [1,2]. From 1916 to 2011, an estimated total of 165 050 000 metric tonnes of TiO_2 pigment were produced worldwide (bulk form and nanoform combined), with a current annual estimated production of more than 6 million tonnes/yr [2]. Reviews of nano-TiO_2 toxicology are available across various evolutionary groups of species [3–8], often summarizing half maximal effective concentration (EC50), half maximal inhibitory concentration (IC50), and median lethal concentration (LC50) values. Nano-TiO_2 is also photoactive and produces reactive oxygen species (ROS) on illumination [9]. Reactive oxygen species can be detrimental to many organisms, causing oxidative damage, cell injury, and ultimately death [10]. Recently, it has been argued that photoactivation of nano-TiO_2 under natural levels of sunlight is sufficient to affect the output of LC50 and EC50 values in standard toxicology tests [11,12]. The majority of studies investigating nano-TiO_2 toxicity did not take photoactivation into account and performed tests either in dark conditions or under indoor commercial artificial lighting that did not simulate natural solar irradiation.

The aim of present study was to derive a phototoxicity ratio between the results of the nano-TiO_2 experiments conducted in the absence of sunlight and conducted in the presence of solar or simulated solar radiation (SSR). To achieve this aim, we searched the literature for studies that included nano-TiO_2 experiments both with and without irradiance under the same experimental setup and otherwise identical conditions. Therefore, the phototoxicity ratio can be used to correct endpoints of the toxicity tests with nano-TiO_2 that were performed in absence of natural sunlight or SSR. Such corrections also may be important for regulators and risk assessors when reviewing previously published data. Another aim is to provide information for improvement of risk assessment of nano-TiO_2. For example, one of the current challenges for conducting risk assessment of nanoparticles such as TiO_2 is lack of consistent toxicity data because of the varieties of materials and test conditions. A phototoxicity ratio derived from existing literature will help to harmonize the toxicity data. Regulatory thresholds for nano-TiO_2 do not exist currently; however, regulation of nanoparticles discharge and monitoring in aquatic environment is anticipated in the future. It is expected that regulators will use the phototoxicity ratio when deriving thresholds for nano-TiO_2 because the majority of the published literature reported toxicity endpoints for nano-TiO_2 in the absence of natural sunlight or SSR.

Of course, the phototoxicity ratio value calculated in present study is not absolutely precise and correct for all environmental conditions and species; it does, however, considerably reduce the possible error of data endpoints obtained in the absence of natural sunlight or SSR. It also mitigates uncertainties in the risk assessment process by taking into account the photoactivation and phototoxicity of nano-TiO_2.

METHODS

A comprehensive literature review was conducted to collect available toxicity endpoints for nano-TiO_2. The literature search

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(September 2014) was performed within 4 databases—Web of Science, Scopus, Google Scholar, and the University of British Columbia library database—using the following keywords in various combinations: titanium dioxide, TiO_2, nanoparticles, phototoxicity, photoactivation, EC50, LC50, IC50, and lowest-observed-effect concentration (LOEC). Abstracts of numerous hits were read, and downloaded papers were checked for useful information. Only papers that reported results under the same environmental conditions for 2 different nano-TiO_2 exposure groups (with and without SSR) in the form of EC50, LC50, IC50, LOEC, or a ratio were selected. Thus, all included data were based on dose–response curves, ensuring the highest possible quality. Such methodology was selected based on the previous study of nano toxicity ratios [13]. The phototoxicity ratio (PR) was calculated in the form of a ratio:

$$\mathrm{PR} = \frac{\mathrm{TiO_2\ LC50,\ EC50,\ IC50,\ LOEC\ \ without\ sunlight\ or\ SSR}}{\mathrm{TiO_2\ LC50,\ EC50,\ IC50,\ LOEC\ with\ sunlight\ or\ SSR}}$$

A phototoxicity ratio greater than 1 means nano-TiO_2 is phototoxic.

In a few isolated cases, results were not given in the form of a number; but it was possible to derive a number based on figures provided. Because the papers reported only irradiance (power of electromagnetic radiation per unit area) intensity and not actual insolation (total amount of solar radiation or SSR energy received on a given surface area during a given time), insolation value was calculated when possible. Insolation was calculated based on the irradiance (W/m^2), actual duration of irradiance (h), and total duration of the toxicity test. In cases in which irradiance intensity was reported in units other than W/m^2, the data were converted for consistency. For the studies in which data existed only in the form of full spectrum insolation, it was important to at least approximate the levels of ultraviolet A (UVA) and ultraviolet B (UVB) used in the studies. At sea level, UVA spectra is accountable for 5.7% of the total sunlight, whereas UVB is accountable for 0.3% of the total sunlight [14]. Thus, UVA and UVB approximations were performed on the studies reporting full spectrum with factors of 0.057 and 0.003 for UVA and UVB, respectively. Such conversions allowed us to determine whether UVA and UVB levels used in the studies were of environmental relevance by comparing the data with published averaged UVA and UVB levels over Europe. The original data of insolation of the full spectrum also are presented.

Because the focus of the present study is environmental relevance, in all cases, data points were excluded from evaluation if testing conditions did not represent environmentally relevant exposure conditions, such as in vitro toxicity tests with cells. Only data from in vivo studies were used. In several studies, nano-TiO_2 toxicity was reported as greater than the highest exposure concentration with no negative toxic effects. In those cases, the highest tested concentration value was used to derive phototoxicity ratio. This procedure was only applied if the reported "greater than" value was from the control TiO_2 group that was not exposed to SSR or sunlight. This might have led to a slight underestimation of the phototoxicity ratio value, which can be seen as a conservative approach. In all cases, the crystal structure of the nano-TiO_2 particles, their primary particle size, and their hydrodynamic diameter were reported, and the data are presented in Table 1. Therefore, collected data are a mixture of both anatase and rutile crystal forms as well as various particle sizes. Ecosystems generally contain a mixture of all sizes and types of crystal structures of anthropogenically introduced nanoparticles with which decision makers have to cope simultaneously; thus, the aim of the phototoxicity ratio is to provide a distinct value within a muddle. The coating of nano-TiO_2 was not taken into account when evaluating phototoxicity of nano-TiO_2, since all of the collected studies have investigated exclusively bare nano-TiO_2.

Data were checked for normality with the Kolmogorov-Smirnov test and were found not to be of normal distribution. Spearman rank correlation was performed between the phototoxicity ratio value and time duration of the reported toxicity test, time duration of irradiance, irradiance intensity, insolation, and the organism taxa to determine whether any of these variables drove the output value. In the case of organism taxa, for the purpose of analysis, a code of 5 different digits was assigned to bacteria, algae, invertebrates, fish, and amphibians. Kruskal-Wallis analysis of variance with a post hoc multiple comparison and/or Mann-Whitney U test were also performed where applicable.

Validation of phototoxicity ratios in correction of toxicity tests endpoint values was performed on data obtained in absence of sunlight or SSR. "True" phototoxicity data summarized in Table 1 (obtained in the presence of sunlight or SSR), served as a control group. Results were log 10 transformed and then statistically compared with either log 10 (data), log 10 (data/median phototoxicity ratio), or log 10 (data/75% phototoxicity ratio quartile). These 3 groups of results originated from the same set of analyzed studies but were obtained in the absence of sunlight or SSR.

RESULTS

The literature search resulted in 25 usable references, from which 62 pairs of data were generated for calculation of a phototoxicity ratio (Table 1). In total, experiments were performed on 20 different species, ranging from bacteria to amphibians. Applied total irradiance was between 0.46 W/m^2 and 231 W/m^2 (mean, 35.63 W/m^2; median, 17 W/m^2), and effective total insolation was between 0.013 Wh/m^2 and 200 Wh/m^2 (mean, 17.44 Wh/m^2; median, 2.83 Wh/m^2). The recalculated and approximated insolation mean and median data are, respectively, 5.64 W/m^2 and 1.7 W/m^2 for UVA, and 0.243 W/m^2 and 0.015 W/m^2 for UVB. The majority of the studies have used the same nano-TiO_2 products (P25 Degussa), resulting in a fairly similar size span of primary particle diameter.

Phototoxicity ratio minimum and maximum values were 0.84 and 16 778, respectively, and mean and median values were 407.5 and 3.7. The discrepancy between the mean and median was caused primarily by the data associated with the Cladocera taxon. When the data were analyzed for susceptibility of bacteria, algae, invertebrates, fish, and amphibians to phototoxicity, the invertebrates were significantly different compared with other groups. Nano-TiO_2 was significantly more toxic to invertebrates after exposure to light compared with other groups, resulting in a greater phototoxicity ratio (Kruskal-Wallis followed by post hoc multiple comparison). On the other hand, the Spearman rank correlation test was not statistically significant for phylogeny and phototoxicity ratio (decreased or increased phototoxicity of nano-TiO_2 from species on the lower organism stadium, such as bacteria, toward more complex organisms, such as amphibians). Also, there was no correlation between phototoxicity ratio and irradiation intensity, duration of irradiation, or received insolation.

Table 1. Review of nano-titanium dioxide (TiO_2) phototoxicity to various species

Organism	TiO_2 primary particle size (nm)[a]	Hydrodynamic diameter of TiO_2 (nm)	Endpoint	Control group EC50; IC50; LC50; LOEC (mg/L)		Experimental group EC50; IC50; LC50; LOEC (mg/L)			Irradiance (W/m^2)	Test duration (h)	Irradiance duration (h:min)	Full spectrum insolation (Wh/m^2)	UVA insolation (Wh/m^2)	UVB insolation (Wh/m^2)	PR	Ref.
				Dark	Indoor light	UVA	UVB	Full spectrum								
Aeromonas hydrophilla	81 A	NA	EC50	25[b]				2.5[b]	200	2	2:00	200.00	11.4	0.6	10.0	[26]
Aeromonas hydrophilla	50–120 A	299–666	IC50	100				40		1/3					2.5	[27]
Aeromonas hydrophilla	20–50 A	253–608	IC50	100				50		1/3					2.0	[27]
Aeromonas hydrophilla	50–130 A	236–618	IC50	100				60		1/3					1.7	[27]
Aeromonas hydrophilla	70–200 A	279–427	IC50	100				100		1/3					1.0	[27]
Aeromonas hydrophilla	15–25 A/R	401–872	IC50	100				100		1/3					1.0	[27]
Artemia salina	25 A	1600–2400	EC50		480.7			4.05	6	48	48:00	6	0.342	0.018	118.7	[28]
Artemia salina	25 A/R	1400–3700	EC50		284.8			4.03	6	48	48:00	6	0.342	0.018	70.7	[28]
Bacillus licheniformis	25 A	20–2000	EC50	19.57				5.23							3.7	[29]
Bacillus licheniformis	25 A	20–2000	EC50	17.66				4.98		2	2:00				3.5	[29]
Bacillus subtilis	66 A/R	320	Ratio	X				X		20	6:00				2.5	[30]
Bacillus subtilis	81 A	NA	EC50	25[b]				0.5[b]	200	1	1:00	200.00	11.4	0.6	50.0	[26]
Bacillus subtilis	<50	828	EC50		300	230			8.2	1/3	0:20		2.73	0	1.3	[31]
Bacillus subtilis	<50	828	EC50		300		300		5.68	1/3	0:20	1.89	0	1.89	1	[31]
Caenorhabditis elegans	21 A/R	300–1500	EC50	100				53	231	96	0:30	1.20	0.0686	0.0036	1.9	[32]
Ceriodaphnia dubia	25 A	200–1000	EC50	27.45				8.26		48	16:00				3.3	[33]
Danio rerio	23 A/R	50–1000	LC50	1		0.3			100[c]	96	32:00		33.33	0	3.3	[34]
Danio rerio	21 A/R	1243	Ratio					X	50	168	24:00	7.14	0.4071	0.0214	1.5	[35]
Danio rerio	21 A/R	200–2000	LC50		500	500			17	96	16	2.83	2.83	0	1	[19]
Danio rerio	21 A/R	200–2000	LC50		500	34			17	96	16	2.83	2.83	0	14.7	[19]
Danio rerio	21 A/R	200–2000	LC50		500	135			17	96	16	2.83	2.83	0	3.7	[19]
Danio rerio	21 A/R	200–2000	LC50		500	20.3			17	96	16	2.83	2.83	0	24.6	[19]
Daphnia magna	21 A/R	345	EC50	29.7		1.2			5.6	48	32:00	3.73	3.73	0	24.8	[36]
Daphnia magna	21 A/R	358	EC50	33.6		3.4			5.6	48	32:00	3.73	3.73	0	9.9	[36]
Daphnia magna	21 A/R	1600–3400	LC50	118				0.06	17	48	8:00	2.83	0.1615	0.0085	1967	[37]
Daphnia magna	25 A/R	150–190	LC50		500			0.0298	17	48	8:00	2.83	0.1613	0.0085	16778	[12]
Daphnia magna	<40 A	<112	LC50	500[d]				0.14	47	8	8:00	47	2.6790	0.0141	3597	[38]
Daphnia similis	25 A	580–1020	EC50		1000			750.55	0.46	48	48:00	0.46	0.0262	0.0014	1.3	[28]
Daphnia similis	25 A/R	780–1400	EC50		1000			60.16	0.46	48	48:00	0.46	0.0262	0.0014	16.6	[28]
Daphnia similis	35 R	350	EC50		100	100				48					1	[39]
Daphnia similis	25 A/R	400	EC50		100	7.8				48					12.8	[39]
Dunaliella tertiolecta	<30 A/R	NA	NOEC		7			3	8.6	168	98:00	5.02	0.2860	0.0151	2.3	[40]
Escheria coli	66 A/R	320	Ratio	X				X		20	6:00				1.8	[30]
Escheria coli	42	NA	LC50	583				1.68		1/2	0:30				347	[41]
Escheria coli	<50	828	EC50		300	300			8.2	1/3	0:20	2.73	2.73	0	1	[31]
Escheria coli	<50	828	EC50		300		168		5.68	1/3	0:20	1.89	0	1.89	1.8	[31]
Escheria coli	23 A/R	970	IC50	25				2.7		2	2:00				9.3	[42]
Escheria coli	79 A	461	IC50	25				4.2							6.0	[42]
Escheria coli	15 A	639	IC50	25				9.1							2.7	[42]
Escheria coli	81 A	436	IC50	25				5							5.0	[42]
Escheria coli	15–25 A/R	401–872	IC50	100				5.9		1					16.9	[27]
Escheria coli	70–200 A	279–427	IC50	100				5.3		1					18.9	[27]
Escheria coli	20–50 A	253–608	IC50	100				11.5		1					8.7	[27]
Escheria coli	50–130 A	236–618	IC50	100				29.3		1					3.4	[27]

(*Continued*)

Table 1. (*Continued*)

Organism	TiO_2 primary particle size (nm)[a]	Hydrodynamic diameter of TiO_2 (nm)	Endpoint	Control group EC50; IC50; LC50; LOEC (mg/L)		Experimental group EC50; IC50; LC50; LOEC (mg/L)			Irradiance (W/m^2)	Test duration (h)	Irradiance duration (h:min)	Full spectrum insolation (Wh/m^2)	UVA insolation (Wh/m^2)	UVB insolation (Wh/m^2)	PR	Ref.
				Dark	Indoor light	UVA	UVB	Full spectrum								
Escheria coli	50–120 A	299–666	IC50	100				66.8		1					1.5	[27]
Gammarus fossarum	21 A/R	97		X				X	28.9	168	84:00	14.45	0.8237	0.0434	2[b]	[43]
Hyalella azteca	25 A/R	616–972	LC50		631			29.9	2.2	96	16:00	0.37	0.0209	0.0011	21.1	[44]
Isochrysis galbana	<30 A/R	NA	NOEC		7			1	8.6	168	98:00	5.02	0.2860	0.0151	7.0	[40]
Moina macropoa	21 A/R	298	EC50		3.6	0.0071			1.7	48	48:00	1.7	1.7	0	507	[45]
Moina macropoa	21 A/R	132	EC50		2.8	0.0033			1.7	48	48:00	1.7	1.7	0	848.5	[45]
Moina macropoa	21 A/R	72	EC50		19	0.0372			1.7	48	48:00	1.7	1.7	0	510.8	[45]
Oryzias latipes	21 A/R	1600–3400	LC50	500				8.5	17	48	8:00	2.83	0.1615	0.0085	58.8	[37]
Oryzias latipes	25 A/R	200–2400	LC50		294			2.46	17	96	16	2.83	0.1615	0.0085	119.5	[12]
Pseudokirchneriella subcapitata	21 A/R	486	EC50		2.53	3			82	72	0:01	0.02	0.02	0	0.8	[46]
Pseudokirchneriella subcapitata	21 A/R	486	EC50		2.53		2.95		56.8	72	0:01	0.01	0	0.01	0.9	[46]
Skeletonema costatum	<30 A/R	NA	NOEC		7			7	8.6	168	98:00	5.02	0.2860	0.0151	1	[40]
Thalassiosira pseudonana	<30 A/R	NA	NOEC		7			3	8.6	168	98:00	5.02	0.2860	0.0151	2.3	[40]
Xenopus laevis	32 A	103–1354	NOEC		77.7	77.7			40	336	196:00	23.33	23.33	0	1.0	[47]
Xenopus laevis	10 A	177–666	NOEC		281.2	30.9			40	336	196:00	23.33	23.33	0	9.1	[47]
Xenopus laevis	5 A	39–398	NOEC		90.2	9.5			40	336	196:00	23.33	23.33	0	9.5	[47]
Xenopus laevis	32 A	103–1354	LC50		295.1	268			40	336	196:00	23.33	23.33	0	1.1	[47]
Xenopus laevis	5 A	39–398	LC50		210.2	57.9			40	336	196:00	23.33	23.33	0	3.6	[47]

[a]Anatase (A) or rutile (R).
[b]Derived data based on the presented data.
[c]Calculated as 0.45 m distance; 30% shadow angle; and 250 W light source.
[d]Not in total darkness. Control received 10% of light source.
EC50 = median effective concentration; IC50 = median inhibitory concentration; LC50 = median lethal concentration; LOEC = lowest-observed-effect concentration; UVA = ultraviolet A; UVB = ultraviolet B; PR = phototoxicity ratio; NOEC = no-observed-effect concentration; NA = data are not available.

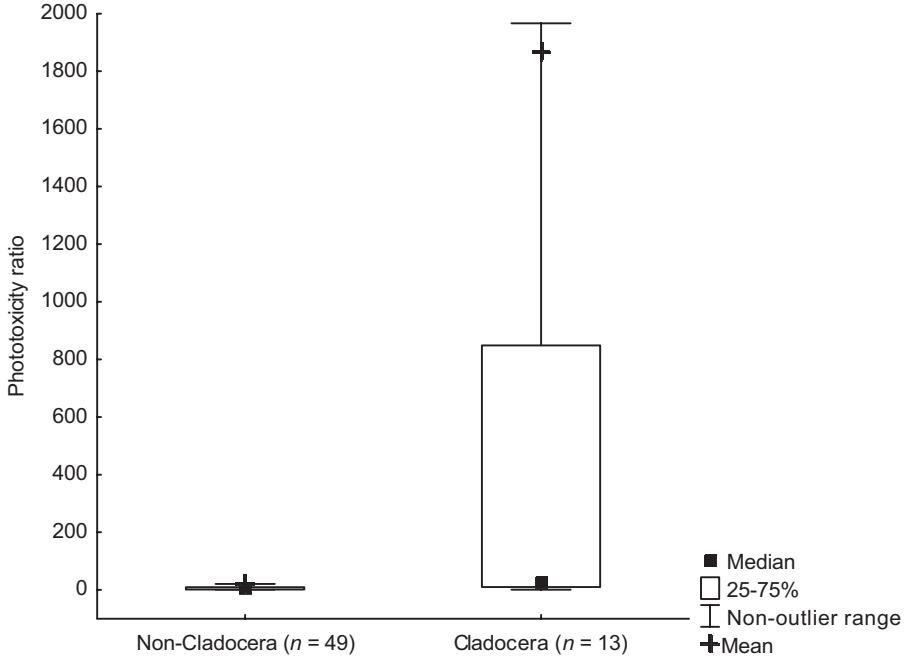

Figure 1. Comparison of phototoxicity ratio values between Cladocera and non-Cladocera species.

Indeed, when exclusive Cladocera data were analyzed against all other taxa (Figure 1), the statistical difference was highly significant (Mann-Whitney U test, $p < 0.01$). Because of the clear need for data segregation, separate descriptive statistics were performed for Cladocera and non-Cladocera phototoxicity ratio values (Table 2). On average, nano-TiO_2 was 20 times more toxic to non-Cladocera and 1867 times more toxic to Cladocera (median values, 3.3 and 24.7, respectively) after illumination.

Significant statistical difference was observed between "true" phototoxicity data and data obtained in the absence of sunlight or SSR (Figure 2). Once the data were corrected by dividing data obtained in the absence of sunlight or SSR with a median phototoxicity ratio or a 75% quartile phototoxicity ratio value, there was no longer statistical difference compared with the data obtained in the presence of sunlight or SSR (Figure 2). However, we do not claim that values for the median phototoxicity ratio and the 75% phototoxicity ratio quartile are definite, because they will change over time as more data points become available from future studies.

DISCUSSION

The fact that the Cladocera taxon was more sensitive to nano-TiO_2 phototoxicity cannot be explained by the intensity of irradiation or received insolation during testing, because such correlation was not statistically significant (Spearman rank correlation test). In Cladocera-related experiments, median irradiation and insolation were even smaller than in experiments with other species. The original publications from which data were derived provided no evidence that Cladocera were exposed to any specific grade, type, or size of nano-TiO_2 particles to which other taxa were not exposed. Ultraviolet sensitivity of the taxon has to be ruled out as well, because appropriate exposure controls to UV were included and no increase in toxicity was detected. Although UV is toxic and lethal to Cladocera at higher exposure doses, numerous protection mechanisms prevent hazardous occurrences at lower doses [15]. Both UV and nano-TiO_2 toxicity are based on ROS, and oxidative stress was indicated in Cladocera exposed to either UV [15,16] or nano-TiO_2 [17]. However, this does not necessarily mean that UV and nano-TiO_2 have the same toxicity mechanism. Whereas generation of ROS and consequently oxidative stress following exposure to UV radiation requires endogenous photosensitizer molecules, generation of ROS by nano-TiO_2 under UV radiation is a direct photochemical process, and the substantial ROS production can readily damage or kill cells or organisms such as Cladocera. Why Cladocera are more sensitive to irradiated nano-TiO_2 remains unclear, and more targeted research is needed. However, one possible explanation for the high sensitivity of Cladocera to nano-TiO_2 phototoxicity is that photoinduced ROS on the surface of Cladocera carapace may interfere with the respiratory gas exchange. In fact, surface attachment of nano-TiO_2 to Cladocera carapace has been observed in previous studies [18,19], and the inner wall of the carapace is a major site of respiratory gas exchange for Cladocera [20].

Sunlight is composed of visible, UV, and infrared spectra. Some of the analyzed studies reported irradiance values exclusively within the UV spectrum, whereas others reported values for the full spectrum. Thus, insolation data also were presented based on reported irradiance spectrum. When the data were segregated into what appeared to be insolation values for

Table 2. Descriptive statistics of phototoxicity ratio (PR) values

PR	Valid *n*	Mean	Median	Minimum	Maximum	25% quartile	75% quartile	Standard deviation	Standard error
Cladocera	12	1867.56	24.75	1.00	16 778.5	9.88	848.48	46 02.74	1276.57
Non-Cladocera	49	20.09	3.33	0.84	347.02	1.50	9.49	54.50	7.79

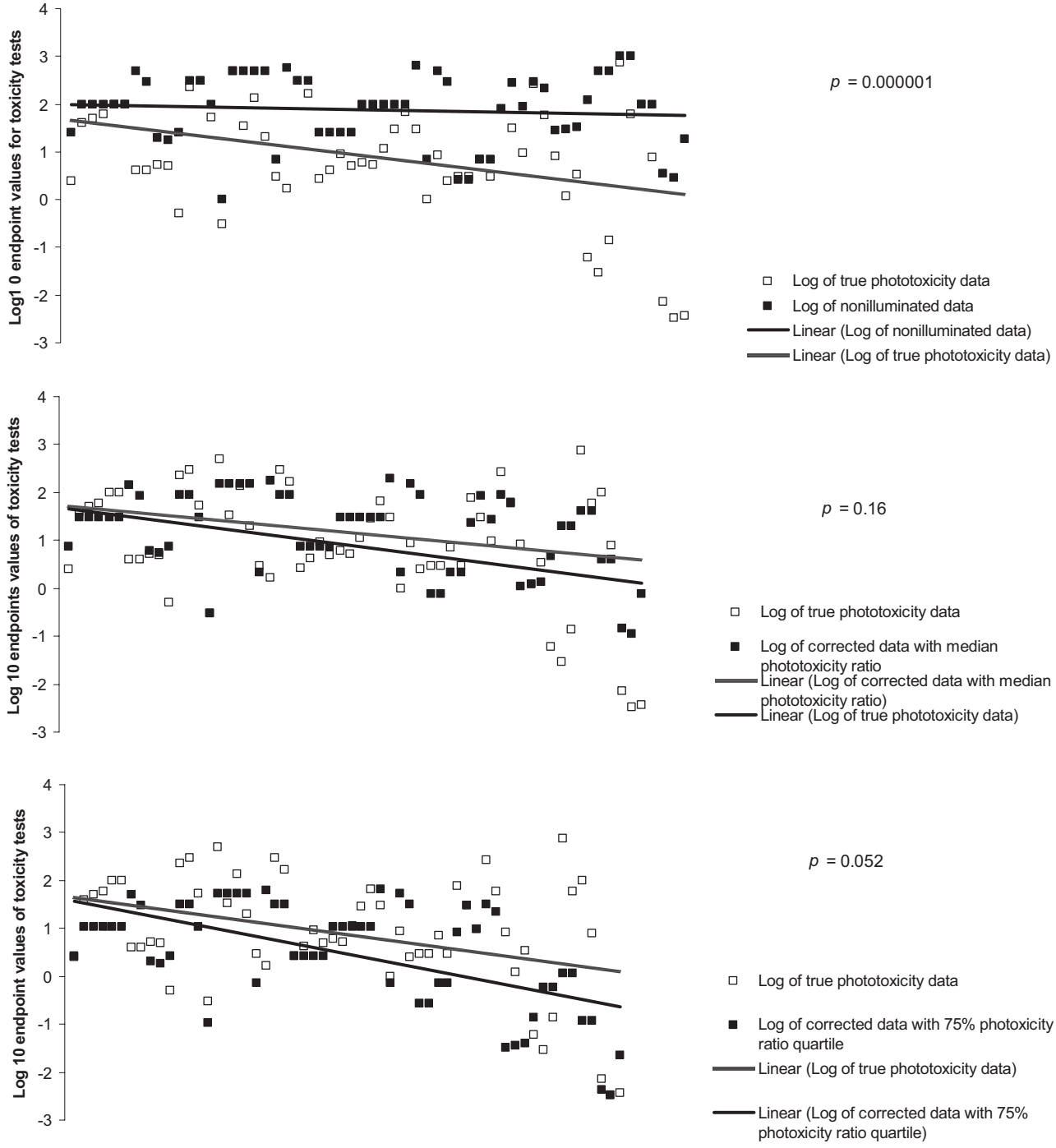

Figure 2. Comparison of uncorrected data (top), corrected data with median phototoxicity ratio (middle), and corrected data with 75% phototoxicity ratio quartile (bottom) obtained in the absence of sunlight or simulated solar radiation (SSR) with true data obtained in the presence of sunlight or SSR. Cladocera and non-Cladocera data were corrected separately with the group corresponding median or 75% quartile values.

the full spectrum, the mean insolation was 25.9 Wh/m^2, and the median was 5 Wh/m^2. The studies that supposedly only reported values for the UV spectrum had a mean insolation of 8.72 Wh/m^2, with a median of 2.83 Wh/m^2. For the purpose of comparison, a solar constant (irradiance of the sun when positioned at 1 astronomical unit compared with Earth at zenith) measured at the outer surface of Earth's atmosphere is approximately 1360 W/m^2 [21]. A significant amount of the solar constant is lost by the time sunlight reaches a location on the Earth's surface, depending on atmosphere, latitude, and time of day. For example, average insolation of the visible spectrum during a decade of measurements over Europe is between 5 Wh/m^2 and 302 Wh/m^2 in winter and between 285 Wh/m^2 and 430 Wh/m^2 in summer [22]. Therefore, both the mean and median (25.9 Wh/m^2 and 5 Wh/m^2, respectively) insolation used in the studies reporting only values for full spectrum are much less than the insolation values over Europe.

The most likely culprits for TiO_2 phototoxicity are UVA and UVB spectrum because those photons would have enough quantum energy (UVA, 3.10–3.94 eV per photon; UVB, 3.94–4.43 eV per photon) [23] versus energy of visible light photon (1.6–3.4 eV) to overcome the band gap. When UVA and UVB

level approximations were performed on the studies reporting full spectrum and combined with studies directly reporting UVA and UVB, mean and median values were 5.64 W/m^2 and 1.7 W/m^2, respectively, for UVA and 0.243 W/m^2 and 0.015 W/m^2, respectively, for UVB. The actual UV spectrum insolation over Europe is, on average, 0.7 Wh/m^2 to 37.7 Wh/m^2 in winter and 34 Wh/m^2 to 64.2 Wh/m^2 in summer for UVA; for UVB, the average is 0.001 Wh/m^2 to 1.08 Wh/m^2 in winter and 0.77 Wh/m^2 to 2.05 Wh/m^2 in summer [22]. The mean and the median values for UVA and UVB used in toxicity studies are well within the range of UVA and UVB values over Europe [22]. Therefore, the current experimental setups represent realistic and natural conditions, and the obtained results should not be doubted. Thus, levels used in experimental setups are credible for the purpose of risk assessment, since they do not exceed natural conditions. It is important to note, however, that approximation to the UVA and UVB values were based on the assumption that all of the irradiation lamps spectra used in the phototoxicity studies fully corresponded to sunlight spectrum. An early study in 1965 suggested that this might not be the case [24]. Although the technology has advanced significantly over the years, there is no absolute guarantee that all of the studies had the proper irradiation lamps. Furthermore, a significant amount of irradiation at sea level altitude is lost because of reflection and adsorption in the water column, according to the Beer-Lambert law

$$\mathrm{I}z = \mathrm{I}_0 e^{-kz}$$

where z is depth, e is natural logorithm, k is attenuation coefficient, and I_0 is the energy of the sunlight at the surface of the water. Although attenuation in pure water might not affect energy of UV light, reflectance of the water surface may, thus reducing the actual UV energy to which aquatic organisms are exposed. However, an opposite effect may occur in shallow waters because of strong scattering of light, thus multiplying the UV exposure levels [25]. Shading effects of macrophyte vegetation may also affect the level of available light. Therefore, although UV insolation levels currently used in nano-TiO_2 phototoxicity studies are credible at the water surface level, it is still not clear whether they are credible for risk assessment below the water surface. The fact that different studies used different exposure time and different irradiances only suggest that current scientific community does not really have a standardized toxicity test to check for the phototoxicity effects of nanoparticles. Therefore, we strongly recommend that universal agreement on irradiation time and irradiance in a standard nanomaterial phototoxicity test is necessary.

A validation test of phototoxicity ratio correction (Figure 2) showed that after correction with a median phototoxicity ratio value the corrected data are no longer statistically different from the real data obtained in the presence of sunlight or SSR. Data correction for the 75% quartile of the phototoxicity ratio was still not significantly different from the real data ($p = 0.052$) but in general generated much lower endpoints (higher toxicity), as expected. However, the value of 75% quartile application is that, compared with the median phototoxicity ratio, it can more successfully prevent false toxicity underestimation. The use of phototoxicity ratio does not mean that the newly corrected data are the true representation of endpoints from toxicity tests, but rather that they are likely as close as possible. The true correction of data can be achieved only by defining a function through regression analysis. However, because many variables— such as particle size, hydrodynamic diameter, crystal structure, illumination time, irradiance, insolation, species, and organic matter content in test media—will likely influence the phototoxicity of nano-TiO_2 (even if their effect is not statistically significant, they will contribute certain percentage of variability), generating such a function will be difficult. In addition, its use in practice will likely not be feasible. Therefore, the use of a phototoxicity ratio is an oversimplified method that can provide an approximate correction with lots of versatility.

One recent study [13] deployed a similar methodology to the present phototoxicity ratio approach to determine which is more toxic in the environment, nanosized or dissolved metals. Toxicity ratio was calculated between median lethal dose, LC50, EC50, and IC50 values of dissolved and nanoparticulated metals to provide corrections for threshold values in existing regulatory standards. Therefore, the ratio metric approach—whether toxicity ratio, phototoxicity ratio, or nano-ratio—is an inexpensive, straightforward method that mitigates uncertainties for the purpose of risk assessment and management, assuming enough literature is available.

In conclusion, the present study found that nano-TiO_2 is phototoxic to aquatic species, because the phototoxicity ratio values were substantially greater than 1 for the majority of analyzed studies. Existing literature on the subject is likely credible for the purpose of risk assessment because the insolation levels used in experimental setups did not exceed UV levels under natural conditions at the water surface. A significant difference was observed between the phototoxicity ratios of 2 analyzed groups: aquatic species belonging to order Cladocera, and all other aquatic species. The order Cladocera is very sensitive and prone to nano-TiO_2 phototoxicity, at least in laboratory-based toxicity tests. A median phototoxicity ratio value and a 75% quartile were chosen as the most practical approach for correcting nano-TiO_2 toxicity endpoints obtained in the absence of sunlight or SSR. Using a median phototoxicity ratio value in correction is a more conservative approach, whereas using the 75% quartile lowers the chance of underestimating toxicity and may be favored by risk assessors when analyzing previously published data. The values for the phototoxicity ratio are not definite and may change as more data become available in the future.

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Data Availability—All of the data used for calculation and statistical analysis are presented in Table 1 of the present study, with corresponding References.

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