RESEARCH ARTICLE

Genotoxicity assessment of triclocarban by comet and micronucleus assays and Ames test

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Abstract

The widespread usage and ubiquitous distribution of triclocarban (3,4,4′-trichlorocarbanilide, TCC) have raised public concerns about its health effects. At present, there is little information about the genotoxicity of TCC. In this study, we used a battery of genotoxicity testing methods including salmonella reverse mutation test (Ames test), comet assay and micronucleus assay to detect the effects of TCC on gene mutation, DNA breakage, and chromosome damage. The results of Ames test showed that TCC at 0.1–1000 μg/plate did not significantly increase the number of revertant colonies in the four standard *Salmonella typhimurium* strains, i.e., TA97, TA98, TA100, and TA102, when compared to the vehicle control. The results from comet assay demonstrated that exposure to 5, 10, or 15 μM TCC for 24 h did not significantly increase the percentage of comet cells, tail length (TL), DNA in tail (T DNA%), or olive tail moment (OTM) in keratinocyte HaCaT and hepatic L02 cells. Moreover, TCC did not markedly enhance the frequency of micronucleated cells or micronuclei in HaCaT and L02 cells in the micronucleus assay. Taken together, the results indicated that TCC did not exhibit any genotoxic effects. Our study provides additional information for the safety profile of TCC.

Keywords Triclocarban · Genotoxicity · DNA breakage · Chromosome damage · Mutagenicity · Risk assessment

Introduction

Triclocarban (3,4,4′-trichlorocarbanilide, TCC) is a broad-spectrum antimicrobial agent that is commonly added to personal care products (Ribado et al. 2017). Since 1957, TCC has been widely applied around the world on a large scale, (Zhu et al. 2019; Gasperi et al. 2014; Musee 2018), and annual consumption exceeds 500 and 1000 tons in the USA and China, respectively (Halden 2014; Zhao et al. 2013). Of note, the US Food and Drug Administration (FDA) banned the use of TCC in over-the-counter hand soaps in 2016 due to the fact that TCC-containing soaps were not found to provide any additional skin-sanitizing benefits (FDA 2016). However, TCC is still approved to be added in numerous products including toothpaste, cosmetics, deodorants, and medical disinfectants in the USA

(FDA 2016). Due to the high consumption and chemical stability, TCC has been reported to be extensively present in the ecological environment (Healy et al. 2017; Satyro et al. 2017; Lozano et al. 2018). More importantly, TCC is frequently detected in human blood and urine (Wei et al. 2017; Ye et al. 2016), suggesting a widespread TCC exposure in human populations. This has given rise to great concerns about the health effects of TCC (Halden et al. 2017).

TCC was initially thought to have an acceptable human safety profile in personal hygiene products (Birch et al. 1978). However, recent studies have shown that TCC has several potential risks including disrupting endocrine function, along with reproductive and developmental toxicities (Huang et al. 2014; Enright et al. 2017; Rochester and Bolden 2017; Vimalkumar et al. 2019). These findings suggest that the health effects of TCC need to be reassessed. Among many health effects, the genotoxicity of chemicals is the most important adverse effect on both the human and ecological environment. However, there are few reports on the genotoxicity of TCC, and there are some contradictions in these limited studies. To date, the mutagenicity of TCC has only been evaluated in the last century in two salmonella reverse mutation assays (Ames tests) which were summarized in

a published review paper (SCCP European Commission 2004). No increases in gene mutation were observed in the tested strains, indicating that TCC did not exhibit mutagenic effects. Furthermore, the effect of TCC on chromosomes has been studied in Chinese hamster ovary cells, and the results showed that TCC did not induce chromosomal aberrations (SCCP European Commission 2004). However, recent studies have shown that TCC caused DNA damage in aquatic organisms and human breast cancer cells assessed by comet assays (Gao et al. 2015; Han et al. 2016; Sood et al. 2013). It should be noted that the strains used in the previous Ames tests for TCC's mutagenicity were not the recommended standard combination according to the Economic Cooperation and Development (OECD) guideline (OECD 1997; SCCP European Commission 2004). To date, the effect of TCC on DNA damage has been studied in the human breast cancer MCF7 cell line (Sood et al. 2013), whereas other important human cell lines have not been investigated. In addition, the potential impact of TCC on chromosome damage has not been studied in human cells. Thus, in this study, we aim to detect the effects of TCC on three different types of genetic endpoints, i.e., gene mutation, DNA breakage, and chromosome damage.

Materials and methods

TCC, salmonella strains, and cell cultures

Four standard *Salmonella typhimurium* strains TA97, TA98, TA100, and TA102, which originally came from Ames Lab, were provided by the Sichuan University Analytical & Testing Center (Chengdu, China). Human keratinocyte HaCaT cell and hepatic L02 cell lines were purchased from KeyGen Bio-technology (Nanjing, China) and China Center for Type Culture Collection (Wuhan, China), respectively. Cells were routinely cultured in high-glucose Dulbecco's modified Eagle medium (DMEM, Gibco Life Technologies, Grand Island, NY, USA) containing 10% (v/v) fetal bovine serum and antibiotics (100 μg/ml streptomycin and 100 unit/ml penicillin). TCC (Meilun Biological Technology Co. Ltd., Dalian, China; purity, ≥ 98%; lot number, M0502A) was prepared by dissolving in dimethylsulfoxide (DMSO) at 10 mM as the stock solution and was further diluted to the desired concentrations with DMEM for each specific assay. The final concentration of DMSO in this study did not exceed 0.2% (v/v), and this concentration has been reported to exert no additional adverse effects on cell growth (Li et al. 2018).

Ames test

The Ames test was performed according to the previous report (Maron and Ames 1983). The mutagenicity of TCC at various concentrations was detected in four standard *Salmonella typhimurium* TA97, TA98, TA100, and TA102 strains with or without metabolic activation. In brief, cultures were obtained from a single colony of salmonella strains in master plates and incubated at 37 °C overnight with shaking at 100 rpm continuously. Subsequently, 100 μl cultures and 100 μl TCC (final concentrations at 1000, 100, 10, 1, or 0.1 μg/plate dissolved in DMSO) were added to 2 ml melted top agar supplemented with 0.5 mM histidine and 0.5 mM biotin. In the presence of S9 activation, metabolic mixture containing 5% (v/v) S9 rat liver extract with cofactors was added to the suspension of tested strains. The melted top mixture was then poured onto glucose minimal plates. The plates were incubated at 37 °C for 2 days. The revertant colonies in different plates for each group were then counted manually. Vehicle control (100 μl/plate DMSO) and positive controls were used, respectively, in this study. In the absence of S9, the positive control chemicals tested in the four types of *Salmonella typhimurium* were as follows: 0.2 μg/plate of 2,4,7-trinitro-9-fluorenone for TA97 and TA98 strains, 1.5 μg/plate of sodium azide for TA100 strain, and 0.5 μg/plate of mitomycin C for TA102 strain. In the presence of S9 activation, the positive control chemicals were 10 μg/plate of 2-aminofluorene for TA97, TA98, as well as TA100 strains and 50 μg/plate of 1,8-dihydroxyanthraquinone for TA102 strain (Maron and Ames 1983). The result of mutagenicity for each group was considered positive if the number of revertant colonies reached at least twofold higher than that of the vehicle control.

MTT assay

Cell viability must be preserved for the validity of the comet and micronucleus assays because necrotic cells may result in false positive results in genotoxicity tests (Collins 2004). Therefore, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was used to measure the cytotoxicity of TCC on HaCaT and L02 cells according to the method described by Gu et al. (2015). Based upon our preliminary experiment, 2.5, 5, 10, 15, 20, and 25 μM TCC were selected in this study. Briefly, HaCaT and L02 cells were seeded at a density of 1×10^4 cells per well in 96-well plates. In the second day, cells were exposed to TCC at the desired concentrations for 24 h. The medium was removed, and 100 μl MTT solution (final concentration at 0.5 mg/ml) was added to each well. Then cells were maintained in a 37 °C incubator for additional 4 h. MTT was reduced to formazan by the succinate dehydrogenase system in viable cells. Subsequently, MTT solution was replaced with DMSO to dissolve the formed formazan crystals. Optical density for each well in the 96-well plate was measured using a microplate spectrophotometer (Multiskan™ GO, Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 570 nm absorbance. Cell viability for each treatment group was calculated and expressed as

"percentage of cell viability (%)" in comparison with the vehicle control (0.2% v/v DMSO).

Alkaline comet assay

The alkaline comet assay is the most sensitive method to detect DNA single-stranded breakage. In this study, we performed the alkaline comet assay to detect whether TCC could induce DNA breakage according to the protocol used in our own lab (Gu et al. 2015). Based upon MTT results, concentrations of 5, 10, and 15 μM TCC were selected in the comet assay to preserve cell viability. Treatment with potassium dichromate at 1 mM for 4 h was used as the positive control, while treatment with 0.2% (v/v) DMSO was set as the vehicle control. Briefly, HaCaT and L02 cells were seeded at a density of 1×10^5 cells per well in 12-well plates prior to the treatment with 5, 10, or 15 μM of TCC for 24 h. Cell suspension was then collected and added into 0.65% low-melting agarose at approximately 37 °C. The mixture was immediately spread onto solidified 0.8% normal-melting agarose in the slide and covered with a coverslip. After solidification, the coverslip was gently removed, and the slides were placed in the lysis buffer ($pH = 10$) containing 10 mM Tris, 100 mM EDTA, 2.5 M NaCl, 1% Triton X-100, and 10% DMSO at 4 °C for 1 h in the dark. The slides were then gently washed with purified water and immersed in the electrophoresis buffer (1 mM EDTA, 300 mM NaOH, pH 13) protected from light for 30 min to allow DNA unwinding. After electrophoresis at 0.75 V/cm for 30 min, each slide was stained with 20 μl ethidium bromide at 20 μg/ml and immediately viewed under a fluorescence microscopy (Eclipse TiU, Nikon Corp., Tokyo, Japan). For each group, the percentage of comet cells was calculated based on 100 randomly selected cells. The extents of DNA damage, demonstrated by tail length (TL), DNA in tail (T DNA%), and olive tail moment (OTM), were further quantified by Comet Assay Software Project.

Micronucleus assay

Micronucleus assay was performed to examine the effect of TCC on the chromosome integrity according to Chen's description (Chen et al. 2015). HaCaT and L02 cells were seeded into 6-well plates at a density of 10^6 cells per well and allowed to attach. Cells were then dosed with 5, 10, or 15 μM TCC for 24 h. DMSO (0.2% v/v) and mitomycin C (MMC) at 1 μg/ml were used as the vehicle and positive control, respectively. Subsequently, cell suspension was collected and lyzed using potassium chloride mild hypotonic solution (75 mM). Cells were fixed in methanol-glacial acetic acid (3:1, v/v) solution and subjected to centrifugation at 1000 rpm for 3 min. The fixation was performed three times. Cells were then resuspended in methanol/glacial acetic acid (99:1, v/v), and the cell suspension was placed onto clean glass slides. Each slide was stained with 20 μl acridine orange at 40 μg/ml, covered with a coverslip, and immediately viewed under a fluorescence microscope. For each group, 1000 cells were chosen randomly, and the number of cells with micronucleus was counted: frequency of micronucleated cells (FMNC) = (micronucleated cells/1000 cells) × 1000‰ and frequency of micronuclei (FMN) = (total number of micronuclei/1000 cells) × 1000‰.

Statistical analysis

Data were calculated from three independent experiments and presented as mean ± standard deviation (SD). Comparisons between groups were determined by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc comparison test. Statistically significant difference was considered as $p < 0.05$. Statistical analysis was performed with the Statistical Program for Social Sciences (SPSS) Version 17.0 (SPSS, Inc., Chicago, IL, USA).

Results

TCC was not mutagenic in Ames test

The results of Ames test were illustrated in Table 1. There was no significant difference in the number of revertant colonies between the vehicle control (DMSO) and the spontaneous mutation group (0 μg/plate TCC). TCC at 1000 μg/plate completely killed all the tested Salmonella strains. TCC at 100 μg/plate significantly decreased the number of revertant colonies due to its antibacterial property as well. However, exposure to TCC at 1 and 0.1 μg/plate did not exhibit any inhibitory effects on the growth of Salmonella strains in the presence or absence of S9, indicating that these concentrations were tolerant by the bacterium. Moreover, TCC at all tested concentrations did not significantly increase the number of revertant colonies in TA97, TA98, TA100, and TA102 strains with or without S9 activation. In contrast, the positive control chemicals markedly increased the corresponding mutant counts over the vehicle control group in each Salmonella strain, demonstrating the sensitivity of our testing system. Taken together, these results indicated that TCC did not induce gene mutation in prokaryotic cells in Ames test.

Cytotoxicity of TCC detected by MTT assay

Considering the fact that TCC is absorbed through dermal contact and is mainly metabolized by the liver in humans (SCCP European Commission 2004), we chose human keratinocyte HaCaT and hepatocyte L02 cells as the cell models in this study. In order to determine the appropriate concentrations for the comet and micronucleus assays, the cytotoxicity of TCC on the tested cell lines was detected by

Table 1 Ames test results of triclocarban

Group	Dose (μg/plate)	S9	Number of colonies/plate (mean ± SD)			
			TA97	TA98	TA100	TA102
Triclocarban	0	–	113.3 ± 14.6	37.7 ± 3.1	148.7 ± 18.5	259.3 ± 19.0
	0.1	–	114 ± 18.4	41.3 ± 2.1	155.0 ± 22.3	268.7 ± 13.7
	1	–	112.3 ± 14.0	38.0 ± 9.5	145.3 ± 14.8	271.7 ± 11.6
	10	-	60.0 ± 13.1	28.0 ± 5.6	112.0 ± 15.4	195.7 ± 12.7
	100	-	13.3 ± 7.8*	12.0 ± 3.0*	34.7 ± 6.7*	33.7 ± 5.5*
	1000	–	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*
Vehicle control		–	111.7 ± 11.9	38.3 ± 5.0	143.0 ± 8.2	258.7 ± 23.1
Positive control		–	1196.7 ± 34.0*	1791.7 ± 52.7*	1272.7 ± 91.8*	923.0 ± 40.6*
Triclocarban	0	+	123.7 ± 12.3	36.3 ± 6.0	143.0 ± 17.3	254.7 ± 19.4
	0.1	+	124.7 ± 16.5	35.0 ± 6.1	143.3 ± 24.0	256.7 ± 24.5
	1	+	127.7 ± 9.7	35.3 ± 4.0	148.7 ± 16.5	261.3 ± 23.7
	10	+	71.0 ± 11.3	23.7 ± 3.5	103.3 ± 12.3	233.7 ± 20.6
	100	+	21.3 ± 6.8*	15.3 ± 3.8*	29.0 ± 7.5*	32.7 ± 14.0*
	1000	+	0 ± 0*	0 ± 0*	0 ± 0*	0 ± 0*
Vehicle control		+	121.7 ± 12.5	35.7 ± 3.2	136.7 ± 7.0	252.7 ± 21.5
Positive control		+	1222.7 ± 120.7*	1857.3 ± 37.0*	1236.0 ± 80.9*	857.7 ± 69.1*

Three independent assays were performed, and values were expressed as the mean ± SD

"*" $p < 0.05$ versus vehicle control group

MTT assay. HaCaT and L02 cells were treated with TCC at various doses (0–25 μM) for 24 h. The results showed that TCC exhibited inhibitory effects on the two human cell lines in a concentration-dependent manner (Fig. 1a, b). TCC at 5, 10, and 15 μM reduced the cell viability of HaCaT cells to 85.1%, 80.6%, and 72.9% when compared to the vehicle control (Fig. 1a). Meanwhile, 5, 10, and 15 μM TCC caused 16.1%, 23.8%, and 28.9% mortality in L02 cells in comparison with the control (Fig. 1b). To avoid the false positive results caused by necrotic cells in genotoxicity tests, graded concentrations of 5, 10, and 15 μM TCC were chosen for the comet and micronucleus assays to ensure the cell viability up to 70%.

TCC did not induce DNA damage in the comet assay

HaCaT and L02 cells were treated with TCC at 5, 10, or 15 μM for 24 h prior to the comet assay. The results showed that TCC did not induce DNA breakage in the two human cell lines at all tested concentrations (Fig. 2a, b). Further quantitative analysis indicated that there were no statistically significant differences in the percentage of comet cells, tail length,
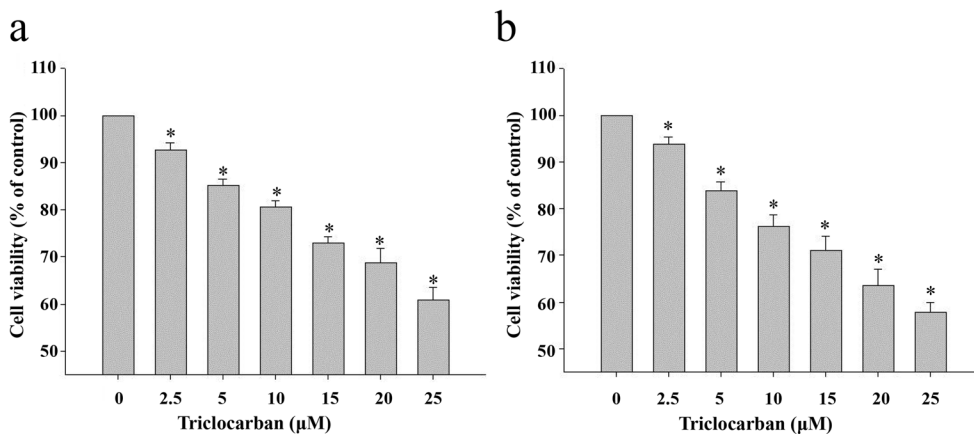

Fig. 1 Effects of triclocarban on cytotoxicity in MTT assay. HaCaT and L02 cells were treated with 0–25 μM triclocarban for 24 h. Cell viability was determined by MTT assay. (a) Effects of various concentrations of triclocarban on cell viability in HaCaT cells. (b) Effects of various concentrations of triclocarban on cell viability in L02 cells. Data are given as mean ± SD. Results are average of three independent experiments. "*" Significant difference between treated and untreated control ($P < 0.05$)

a

DMSO   Potassium Dichromate

5 μM   10 μM   15 μM
Triclocarban   Triclocarban   Triclocarban

b

DMSO   Potassium dichromate

5 μM   10 μM   15 μM
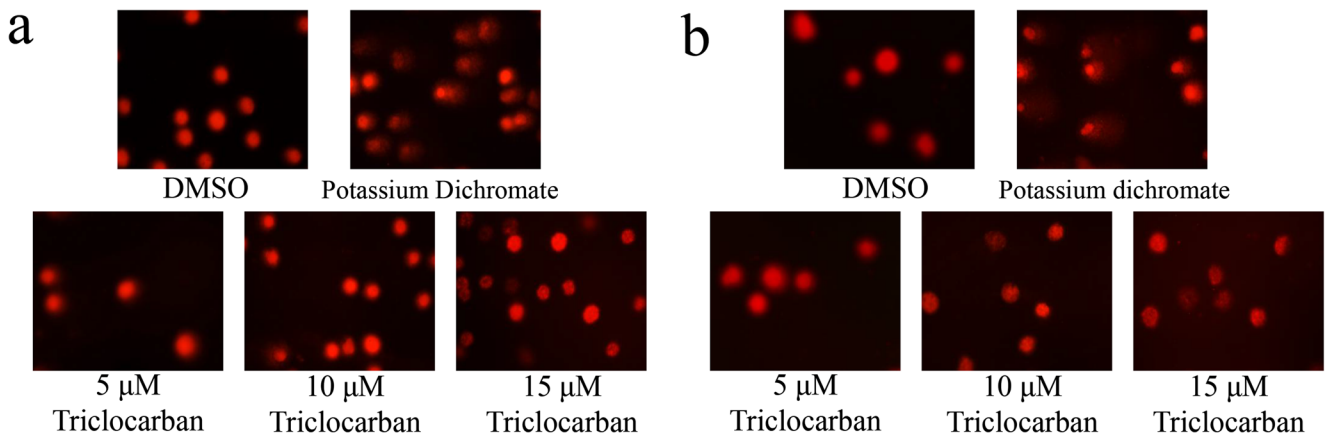Triclocarban   Triclocarban   Triclocarban

Fig. 2 Effects of triclocarban on DNA damage in HaCaT and L02 cells in comet assay. Cells were treated with various concentrations of triclocarban for 24 h, and DNA breakage was determined. Representative images at × 400 magnification from the comet assay are shown. (a) HaCaT cells treated by 5, 10, or 15 μM triclocarban and 1 mmol/L potassium dichromate. (b) L02 cells treated by 5, 10, or 15 μM triclocarban and 1 mmol/L potassium dichromate

DNA in tail, and OTM values between TCC treatment groups and the vehicle control (Table 2). In contrast, the positive control treated with 1 mM potassium dichromate induced DNA damage in HaCaT and L02 cells (Fig. 2a, b, Table 2), demonstrating that the experimental condition was sensitive to detect DNA damage. Taken together, exposure to TCC at 5, 10, or 15 μM for 24 h did not induce DNA damage in both HaCaT and L02 cells measured by the comet assay.

TCC did not cause chromosome damage in the micronucleus test

We further performed micronucleus test to detect the effect of TCC on the chromosome integrity in the two human cell lines. The results showed that treatment with 5, 10, or 15 μM TCC for 24 h did not increase the number of micronuclei in both HaCaT and L02 cells under microscopic examination (Fig. 3a, b). Further quantitative analysis demonstrated that

TCC at all tested concentrations did not significantly increase the frequency of micronucleated cells or induce excessive micronuclei in comparison with the vehicle control (Table 3). However, the micronucleus frequency in the positive control group (1 μg/ml MMC for 24 h) was significantly higher than that of the vehicle control (Fig. 3a, b, Table 3), indicating the sensitivity of our micronucleus assay to detect chromosome damage. Taken together, the results showed that TCC did not exhibit any adverse effects on the chromosome integrity in the two human cell lines in the micronucleus test.

Discussion

To date, studies on the genotoxicity of TCC are incomplete due to the lack of data using a standard combination of *Salmonella typhimurium* strains in Ames test and the absence of examination on essential genotoxic endpoints in

Table 2 Comet assay results of triclocarban

Cell line	Group	Dose (μM)	Percentage of comet cells (%)	Tail length	DNA in tail (%)	Olive tail moment
Hacat	Triclocarban	0	8.00 ± 2.00	3.81 ± 0.30	0.97 ± 0.10	2.73 ± 0.24
		5	8.67 ± 3.79	3.68 ± 0.34	0.99 ± 0.08	2.86 ± 0.27
		10	8.33 ± 1.15	4.05 ± 0.17	1.01 ± 0.04	2.81 ± 0.28
		15	9.33 ± 2.52	3.94 ± 0.08	0.94 ± 0.06	2.77 ± 0.30
	Positive control		$73.00 \pm 4.58^{*}$	$60.05 \pm 6.79^{*}$	$21.56 \pm 3.79^{*}$	$16.51 \pm 2.52^{*}$
L02	Triclocarban	0	7.33 ± 0.58	3.84 ± 0.10	0.95 ± 0.11	2.76 ± 0.30
		5	9.33 ± 2.52	3.86 ± 0.16	0.97 ± 0.11	2.68 ± 0.14
		10	8.67 ± 1.53	3.94 ± 0.19	1.05 ± 0.06	2.87 ± 0.31
		15	9.00 ± 1.00	4.02 ± 0.20	1.02 ± 0.15	2.85 ± 0.32
	Positive control		$70.67 \pm 6.66^{*}$	$57.97 \pm 5.51^{*}$	$19.86 \pm 1.70^{*}$	$16.58 \pm 1.89^{*}$

Three independent assays were performed, and values were expressed as the mean ± SD

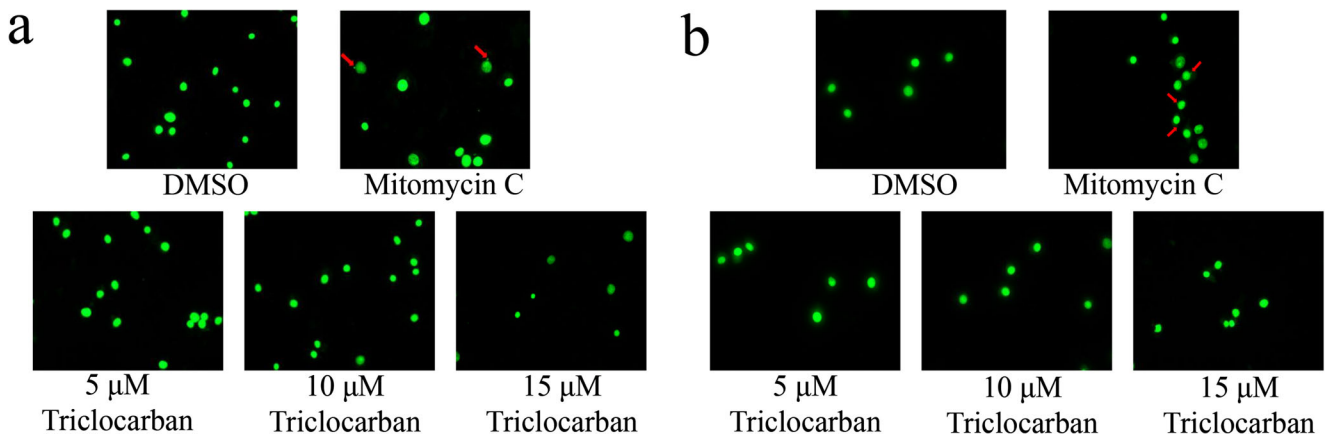"*" $p < 0.05$ versus vehicle control (0 μM triclocarban)

Fig. 3 Effects of triclocarban on chromosome damage in HaCaT and L02 cells in micronucleus assay. Cells were treated with various concentrations of triclocarban for 24 h. The micronuclei were marked with an arrow. (a) Images at × 400 magnification of HaCaT cells treated by 5, 10, or 15 μM triclocarban and 1 μg/ml mitomycin C (MMC). (b) Images of L02 cells treated by 5, 10, or 15 μM triclocarban and 1 μg/ml MMC

appropriate human cell lines. DNA breakage, chromosome damage, and gene mutation are the main types of genetic endpoints in genotoxicity studies (Thybaud et al. 2017). Clearly, a single assay is not sufficient to examine the impacts of TCC on all these endpoints. Thus, we performed Ames test, comet assay, and micronucleus test to detect the effects of TCC on gene mutation, DNA breakage, and chromosome damage. We found that TCC did not exhibit any genotoxic effects under the current experimental conditions.

Ames test is the most commonly used genetic toxicology method to detect gene mutation induced by chemicals in prokaryotic cells (Cimino 2006). Four standard *Salmonella typhimurium* strains TA97, TA98, TA100, and TA102 are recommended in Ames test for detecting three classes of gene mutation (i.e., TA97 and TA98 for frame shifts, TA100 for base-pair substitution, and TA102 for transitions/transversions) (Mortelmans and Zeiger 2000; Maron and Ames 1983; OECD 1997). It has been reported that doses of 8, 40, 200, and 1000 μg/plate TCC are not mutagenic to

TA98, TA100, TA1537, and TA1538 strains (SCCP European Commission 2004). However, the mutagenicity of TCC has not been detected in TA97 and TA102 strains. In fact, TA97 has a specificity similar to that of TA1537 but is more sensitive to various frame shift mutagens, and TA102 is more sensitive to specific mutagens which are inactive in TA1538 (De Flora et al. 1984). Thus, the mutagenicity of TCC was determined in TA97 and TA102 strains in this study. In addition, the doses of TCC (≥ 8 μg/plate) in previous studies may kill the tested strains and diminish the reverse mutation, thereby causing false negative results. Therefore, we used 0.1 and 1 μg/plate TCC in Ames test to reduce the antibacterial effect. TCC at 1000 μg/plate was used as the maximum concentration in this study based on the previous studies (SCCP European Commission 2004). Taken together, our study examined the mutagenicity of TCC at rational concentrations in the standard combination of *Salmonella typhimurium* strains, demonstrating that TCC did not induce gene mutation in Ames test.

Table 3 Micronucleus assay results of triclocarban

Cell line	Group	Dose (μM)	Frequency of micronucleated cells (‰)	Frequency of micronuclei (‰)
Hacat	Triclocarban	0	4.00 ± 1.73	4.00 ± 1.73
		5	3.67 ± 1.15	3.67 ± 1.15
		10	5.00 ± 1.73	5.33 ± 1.53
		15	4.67 ± 1.53	4.67 ± 1.53
	Positive control		34.67 ± 5.86[*]	37.33 ± 3.79[*]
L02	Triclocarban	0	4.33 ± 1.53	4.33 ± 1.53
		5	4.67 ± 1.15	4.67 ± 1.15
		10	5.00 ± 1.73	5.00 ± 1.73
		15	4.67 ± 2.08	5.00 ± 2.65
	Positive control		39.33 ± 6.43[*]	41.33 ± 7.37[*]

Three independent assays were performed, and values were expressed as the mean ± SD

"*" $p < 0.05$ versus vehicle control (0 μM triclocarban)

In this study, the comet assay was performed to detect the genotoxicity of TCC since some non-mutagens could induce DNA damage in humans (Clive 1985). Alkaline comet assay is more sensitive than neutral comet assay and is able to detect single-stranded breaks, double-stranded breaks, and alkaline-labile sites in DNA (Pu et al. 2015). Thus, the alkaline comet assay was used to detect the alterations in DNA in this study. Reactive oxygen species (ROS) are a group of short-lived, highly reactive, oxygen-containing molecules that can induce DNA damage (Srinivas et al. 2019). Although TCC has been found to induce ROS in aquatic species (Xu et al. 2015; Han et al. 2016), this antimicrobial fails to induce ROS in human cells (Simon et al. 2014). This may explain the discrepancy of genotoxic effects of TCC between aquatic organisms and human cells. It has been reported that chronic exposure to 200 nM TCC increases the value of tail moment in human breast cells in the comet assay (Sood et al. 2013). However, we found that treatment with 15 μM TCC for 24 h did not induce DNA breakage in HaCaT and L02 cells, as demonstrated by multiple parameters including the percentage of comet cells, tail length, DNA in tail rate, and OTM value. The discrepancy may be due to the exposure time and the fact that different human cells exhibit different sensitivity to ROS. It is rational to use HaCaT and L02 cells as the cell models in our study because the skin and liver are the targeting tissues for TCC.

Chromosome breakage is a basic genotoxic endpoint in the genotoxicity assessment. Although it is found that TCC cannot induce chromosomal aberrations in Chinese hamster ovary cells (SCCP European Commission 2004), TCC's effect on the chromosome integrity in human cells has not been investigated. Micronucleus tests using human-specific cell models are sensitive to detect chromosome damage and can provide evidence on the genotoxicity of chemicals (Turkez et al. 2017; Miller et al. 1997). Thus, the micronucleus assay was used in this study to examine the effect of TCC on the chromosome integrity in HaCaT and L02 cells. Consistent with the findings in Chinese hamster ovary cells, TCC did not exhibit any genotoxic effects on chromosomes in the two human cell lines. It has been reported that the median concentration of TCC in 91 blood samples from women is 0.048 ng/ml (0.00015 μM) (Wei et al. 2017). TCC at 0.00015 μM in plasma cannot be genotoxic to humans because doses of up to 15 μM TCC failed to induce genotoxic effects in our study.

It is worth mentioning that TCC and triclosan (2,4,4′-trichloro-2′-hydroxydiphenyl ether) are the most two high-production-volume antimicrobials used in a variety of products and are frequently discussed in tandem. Triclosan is a diphenyl ether (with oxygen linking the rings), whereas TCC is a carbanilide where the two aromatic rings are linked by urea (Witorsch and Thomas 2010). Based on the discrepancy of chemical structure, they may have different health effects on humans (Halden 2016). In fact, our previous study has demonstrated that triclosan at 10 μM caused approximately 40% cell death in human HaCaT and L02 cells (Sun et al. 2019), while TCC at 15 μM induced about 30% cell mortality in the same types of cells in this study, indicating that the TCC is less toxic than triclosan. Although the two antimicrobial agents have been reported to cause DNA damage in aquatic species (Wang et al. 2018; Shi et al. 2019), they did not induce DNA or chromosome damage in human normal cells based on our previous study (Sun et al. 2019) and our current research. This notion is also supported by another antibiotic, oxytetracycline, which is widely used for therapeutic purposes in humans and aquaculture. Though oxytetracycline is genotoxic to aquatic species (Rodrigues et al. 2017), it has been well-documented to have no genotoxic potential in humans (Kersten et al. 1999). All these indicate that aquatic organisms and humans do not respond to the genotoxic effect of chemicals in the same way.

TCC has been proposed as an estrogen-like chemical, acting as estrogen receptor (ER) agonists to downregulate the expression of endogenous ERα (Huang et al. 2014). Moreover, TCC is also considered to pose potential developmental and reproductive toxicities (Rochester and Bolden 2017). We found that TCC was not genotoxic to the two human cell lines and Salmonella strains in this study. However, other adverse health effects of TCC cannot be ruled out and need to be further studied.

Conclusions

In summary, TCC at concentrations of 0.1–1000 μg/plate did not induce gene mutation in the four standard *Salmonella typhimurium* strains TA97, TA98, TA100, and TA102 in Ames test. Furthermore, the comet assay and micronucleus test showed that exposure to 5, 10, or 15 μM TCC for 24 h did not significantly induce DNA damage or increase the frequency of micronucleus in HaCaT and L02 cells, respectively. Our results indicated that TCC did not exhibit any genotoxic effects under the experimental conditions. TCC is now still widely used, so researches on the health effects associated with TCC are needed in the future.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Birch CG, Hiles RA, Eichhold TH, Jeffcoat AR, Handy RW, Hill JM, Willis SL, Hess TR, Wall ME (1978) Biotransformation products of 3,4,4′-trichlorocarbanilide in rat, monkey, and man. Drug Metab Dispos 6:169–176

Chen C, Jiang X, Lai Y, Liu Y, Zhang Z (2015) Resveratrol protects against arsenic trioxide-induced oxidative damage through maintenance of glutathione homeostasis and inhibition of apoptotic progression. Environ Mol Mutagen 56:333–346. https://doi.org/10.1002/em.21919

Cimino MC (2006) Comparative overview of current international strategies and guidelines for genetic toxicology testing for regulatory purposes. Environ Mol Mutagen 47:362–390. https://doi.org/10.1002/em.20216

Clive D (1985) Mutagenicity in drug development: interpretation and significance of test results. Regul Toxicol Pharmacol 5:79–100

Collins AR (2004) The comet assay for DNA damage and repair: principles, applications, and limitations. Mol Biotechnol 26:249–261. https://doi.org/10.1385/mb:26:3:249

De Flora S, Camoirano A, Zanacchi P, Bennicelli C (1984) Mutagenicity testing with TA97 and TA102 of 30 DNA-damaging compounds, negative with other Salmonella strains. Mutat Res 134:159–165

Enright HA, Falso MJS, Malfatti MA, Lao V, Kuhn EA, Hum N, Shi Y, Sales AP, Haack KW, Kulp KS, Buchholz BA, Loots GG, Bench G, Turteltaub KW (2017) Maternal exposure to an environmentally relevant dose of triclocarban results in perinatal exposure and potential alterations in offspring development in the mouse model. PLoS One 12:e0181996. https://doi.org/10.1371/journal.pone.0181996

FDA (2016) Safety and effectiveness of consumer antiseptics; topical antimicrobial drug products for over-the-counter human use. Final rule. Fed Regist 81:61106–61130

Gao L, Yuan T, Cheng P, Bai Q, Zhou C, Ao J, Wang W, Zhang H (2015) Effects of triclosan and triclocarban on the growth inhibition, cell viability, genotoxicity and multixenobiotic resistance responses of Tetrahymena thermophila. Chemosphere 139:434–440. https://doi.org/10.1016/j.chemosphere.2015.07.059

Gasperi J, Geara D, Lorgeoux C, Bressy A, Zedek S, Rocher V, El Samrani A, Chebbo G, Moilleron R (2014) First assessment of triclosan, triclocarban and paraben mass loads at a very large regional scale: case of Paris conurbation (France). Sci Total Environ 493:854–861. https://doi.org/10.1016/j.scitotenv.2014.06.079

Gu S, Chen C, Jiang X, Zhang Z (2015) Resveratrol synergistically triggers apoptotic cell death with arsenic trioxide via oxidative stress in human lung adenocarcinoma A549 cells. Biol Trace Elem Res 163:112–123. https://doi.org/10.1007/s12011-014-0186-2

Halden RU (2014) On the need and speed of regulating triclosan and triclocarban in the United States. Environ Sci Technol 48:3603–3611. https://doi.org/10.1021/es500495p

Halden RU (2016) Lessons learned from probing for impacts of triclosan and triclocarban on human microbiomes. mSphere 1. doi: https://doi.org/10.1128/mSphere.00089-16

Halden RU, Lindeman AE, Aiello AE, Andrews D, Arnold WA, Fair P, Fuoco RE, Geer LA, Johnson PI, Lohmann R, McNeill K, Sacks VP, Schettler T, Weber R, Zoeller RT, Blum A (2017) The Florence Statement on triclosan and triclocarban. Environ Health Perspect 125:064501. https://doi.org/10.1289/ehp1788

Han J, Won EJ, Hwang UK, Kim IC, Yim JH, Lee JS (2016) Triclosan (TCS) and triclocarban (TCC) cause lifespan reduction and reproductive impairment through oxidative stress-mediated expression of the defensome in the monogonont rotifer (Brachionus koreanus). Comp Biochem Physiol C Toxicol Pharmacol 185-186:131–137. https://doi.org/10.1016/j.cbpc.2016.04.002

Healy MG, Fenton O, Cormican M, Peyton DP, Ordsmith N, Kimber K, Morrison L (2017) Antimicrobial compounds (triclosan and triclocarban) in sewage sludges, and their presence in runoff following land application. Ecotoxicol Environ Saf 142:448–453. https://doi.org/10.1016/j.ecoenv.2017.04.046

Huang H, Du G, Zhang W, Hu J, Wu D, Song L, Xia Y, Wang X (2014) The in vitro estrogenic activities of triclosan and triclocarban. J Appl Toxicol 34:1060–1067. https://doi.org/10.1002/jat.3012

Kersten B, Zhang J, Brendler-Schwaab SY, Kasper P, Muller L (1999) The application of the micronucleus test in Chinese hamster V79 cells to detect drug-induced photogenotoxicity. Mutat Res 445:55–71

Li X, Gu S, Sun D, Dai H, Chen H, Zhang Z (2018) The selectivity of artemisinin-based drugs on human lung normal and cancer cells. Environ Toxicol Pharmacol 57:86–94. https://doi.org/10.1016/j.etap.2017.12.004

Lozano N, Rice CP, Ramirez M, Torrents A (2018) Fate of triclocarban in agricultural soils after biosolid applications. Environ Sci Pollut Res Int 25:222–232. https://doi.org/10.1007/s11356-017-0433-0

Maron DM, Ames BN (1983) Revised methods for the Salmonella mutagenicity test. Mutat Res 113:173–215

Miller B, Albertini S, Locher F, Thybaud V, Lorge E (1997) Comparative evaluation of the in vitro micronucleus test and the in vitro chromosome aberration test: industrial experience. Mutat Res 392(45–59):187–208

Mortelmans K, Zeiger E (2000) The Ames Salmonella/microsome mutagenicity assay. Mutat Res 455:29–60

Musee N (2018) Environmental risk assessment of triclosan and triclocarban from personal care products in South Africa. Environ Pollut 242:827–838. https://doi.org/10.1016/j.envpol.2018.06.106

OECD (1997) OECD guideline for testing of chemicals: bacterial reverse mutation test. Organization for Economic Cooperation and Development http://www.oecd.org/dataoecd/18/31/1948418.pdf

Pu X, Wang Z, Klaunig JE (2015) Alkaline comet assay for assessing DNA damage in individual cells. Curr Protoc Toxicol 65:3.12.11–3.12.11. https://doi.org/10.1002/0471140856.tx0312s65

Ribado JV, Ley C, Haggerty TD, Tkachenko E, Bhatt AS (2017) Household triclosan and triclocarban effects on the infant and maternal microbiome. EMBO Mol Med 9:1732–1741. https://doi.org/10.15252/emmm.201707882

Rochester JR, Bolden AL (2017) Potential developmental and reproductive impacts of triclocarban: a scoping review. J Toxicol 2017:9679738. https://doi.org/10.1155/2017/9679738

Rodrigues S, Antunes SC, Correia AT, Nunes B (2017) Rainbow trout (Oncorhynchus mykiss) pro-oxidant and genotoxic responses following acute and chronic exposure to the antibiotic oxytetracycline. Ecotoxicology 26:104–117. https://doi.org/10.1007/s10646-016-1746-3

Satyro S, Saggioro EM, Verissimo F, Buss DF, de Paiva Magalhaes D, Oliveira A (2017) Triclocarban: UV photolysis, wastewater disinfection, and ecotoxicity assessment using molecular biomarkers. Environ Sci Pollut Res Int 24:16077–16085. https://doi.org/10.1007/s11356-017-9165-4

SCCP European Commission (2004) Opinion on triclocarban for other uses than as a preservative. *Scientific Committee on Consumer Products* Report No.: SCCP/0851/04

Shi Q, Zhuang Y, Hu T, Lu C, Wang X, Huang H, Du G (2019) Developmental toxicity of triclocarban in zebrafish (Danio rerio) embryos. J Biochem Mol Toxicol 33:e22289. https://doi.org/10.1002/jbt.22289

Simon A, Maletz SX, Hollert H, Schaffer A, Maes HM (2014) Effects of multiwalled carbon nanotubes and triclocarban on several eukaryotic cell lines: elucidating cytotoxicity, endocrine disruption, and reactive oxygen species generation. Nanoscale Res Lett 9:396. https://doi.org/10.1186/1556-276x-9-396

Sood S, Choudhary S, Wang HC (2013) Induction of human breast cell carcinogenesis by triclocarban and intervention by curcumin.

Biochem Biophys Res Commun 438:600–606. https://doi.org/10.1016/j.bbrc.2013.08.009

Srinivas US, Tan BWQ, Vellayappan BA, Jeyasekharan AD (2019) ROS and the DNA damage response in cancer. Redox Biol 25:101084. https://doi.org/10.1016/j.redox.2018.101084

Sun D, Zhao T, Li X, Zhang Z (2019) Evaluation of DNA and chromosomal damage in two human HaCaT and L02 cells treated with varying triclosan concentrations. J Toxic Environ Health A 82: 473–482. https://doi.org/10.1080/15287394.2019.1618758

Thybaud V, Lorge E, Levy DD, van Benthem J, Douglas GR, Marchetti F, Moore MM, Schoeny R (2017) Main issues addressed in the 2014-2015 revisions to the OECD genetic toxicology test guidelines. Environ Mol Mutagen 58:284–295. https://doi.org/10.1002/em.22079

Turkez H, Arslan ME, Ozdemir O (2017) Genotoxicity testing: progress and prospects for the next decade. Expert Opin Drug Metab Toxicol 13:1089–1098. https://doi.org/10.1080/17425255.2017.1375097

Vimalkumar K, Seethappan S, Pugazhendhi A (2019) Fate of triclocarban (TCC) in aquatic and terrestrial systems and human exposure. Chemosphere 230:201–209. https://doi.org/10.1016/j.chemosphere.2019.04.145

Wang F, Xu R, Zheng F, Liu H (2018) Effects of triclosan on acute toxicity, genetic toxicity and oxidative stress in goldfish (Carassius auratus). Exp Anim 67:219–227. https://doi.org/10.1538/expanim.17-0101

Wei L, Qiao P, Shi Y, Ruan Y, Yin J, Wu Q, Shao B (2017) Triclosan/triclocarban levels in maternal and umbilical blood samples and their association with fetal malformation. Clin Chim Acta 466:133–137. https://doi.org/10.1016/j.cca.2016.12.024

Witorsch RJ, Thomas JA (2010) Personal care products and endocrine disruption: a critical review of the literature. Crit Rev Toxicol 40(Suppl 3):1–30. https://doi.org/10.3109/10408444.2010.515563

Xu X, Lu Y, Zhang D, Wang Y, Zhou X, Xu H, Mei Y (2015) Toxic assessment of triclosan and triclocarban on Artemia salina. Bull Environ Contam Toxicol 95:728–733. https://doi.org/10.1007/s00128-015-1641-2

Ye X, Wong LY, Dwivedi P, Zhou X, Jia T, Calafat AM (2016) Urinary concentrations of the antibacterial agent triclocarban in United States residents: 2013-2014 National Health and Nutrition Examination Survey. Environ Sci Technol 50:13548–13554. https://doi.org/10.1021/acs.est.6b04668

Zhao JL, Zhang QQ, Chen F, Wang L, Ying GG, Liu YS, Yang B, Zhou LJ, Liu S, Su HC, Zhang RQ (2013) Evaluation of triclosan and triclocarban at river basin scale using monitoring and modeling tools: implications for controlling of urban domestic sewage discharge. Water Res 47:395–405. https://doi.org/10.1016/j.watres.2012.10.022

Zhu Q, Jia J, Wang Y, Zhang K, Zhang H, Liao C, Jiang G (2019) Spatial distribution of parabens, triclocarban, triclosan, bisphenols, and tetrabromobisphenol A and its alternatives in municipal sewage sludges in China. Sci Total Environ 679:61–69. https://doi.org/10.1016/j.scitotenv.2019.05.059