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The Cytotoxicity of Cyanuric Acid and Certain Derivatives in Vitro

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ABSTRACT

Background
The s-triazine category includes cyanuric acid and its derivatives. They are widely used in industry, mostly in the manufacturing of disinfectants, bleaching agents, and pesticides. According to recent findings, cyanuric acid alone has a low overall cytotoxicity, but when combined with melamine, it has a large unfavorable effect. Nevertheless, assessments of cyanuric acid toxicity against several human cell types have not been carried out utilizing a panel of in vitro experiments. Furthermore, the cytotoxicity and possible anticancer effects of several cyanuric acid derivatives, such as 1,3,5-tris(2-hydroxyethyl) isocyanurate and trichloroisocyanuric acid, are poorly understood.

Aim
To use various cancer cell lines and healthy fibroblasts to examine the toxicity of cyanuric acid, trichloroisocyanuric acid, and 1,3,5-tris(2-hydroxyethyl) isocyanurate.

Materials and Procedures
The in vitro cytotoxicity of cyanuric acid, trichloroisocyanuric acid, and 1,3,5-tris(2-hydroxyethyl) isocyanurate was assessed using MTT, Neutral Red, and Clonogenic assays. Three cancer cell lines (HeLa, A549, and CaOV) as well as healthy human fibroblasts were used to test various doses of the chemicals. We computed and compared IC50 values. Following exposure to cyanuric acid, trichloroisocyanuric acid, and 1,3,5-tris(2-hydroxyethyl) isocyanurate, intracellular ATP measurements and microscopic assessments of cellular shape were carried out.

Findings
Trichloroisocyanuric acid (TCIC) showed the highest in vitro cytotoxicity of the three test agents. It's interesting to note that MTT experiments showed that TCIC's harmful effects were mostly focused on cancer cells that had mitochondrial dysfunction. The presence of modest concentrations of triazine fully suppressed the clonogenicity of cancer cell lines. Both cancer cells and normal fibroblasts' shape and intracellular ATP levels were affected by TCIC exposure, however the effects were more pronounced in cancer cells.

Conclusion
The studied chemicals' cytotoxicity is arranged as follows: 1,3,5-tris(2-hydroxyethyl) isocyanurate > cyanuric acid > trichloroisocyanuric acid. The anticancer action of trichloroisocyanuric acid is selective.

Keywords
Cyanuric acid; Trichloroisocyanuric acid; 1,3,5-tris(2-hydroxyethyl) isocyanurate; *In vitro* cytotoxicity; Cancer cell lines; Fibroblasts.

Abbreviations
ATCC: American Type Culture Collection; ATP: Adenosine triphosphate; CA: Cyanuric acid; CSP: cyclosporine; IC_{50}: 50% inhibiting concentration; NBIMCC: National Bank for Industrial Microorganisms and Cell Cultures; TCIC: Trichloroisocyanuric acid; THIC: 1,3,5-tris(2-hydroxyethyl) isocyanurate.

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INTRODUCTION

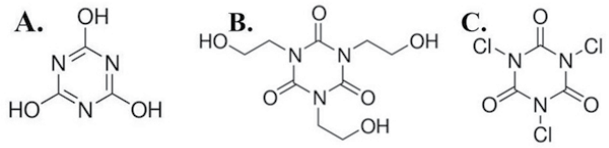
The heterocyclic molecules cyanuric acid (CA) and its derivatives have a symmetric 1,3,5-triazine core. The chemical, pharmaceutical, and textile industries use them extensively.1,2 The uses of cyanuric acid (1,3,5-triazine-2,4,6-triol; 2,4,6-trihydroxy-1,3,5-triazine (Figure 1, A.)) range from disinfecting buildings, textiles, and vessels to various organic synthesis fields (such as the production of glues, antioxidants, pesticides, herbicides, resins, bleaching agents, and antineoplastic drugs).1,3,4 Humans can be exposed to cyanuric acid by drinking water that has been treated from surface water or by eating fish, which can accumulate the chemical.5,6 It has been demonstrated that CA has low acute toxicity in mammals. Additionally, in vitro studies on kidney cell lines exposed to CA in humans, dogs, and cats have shown low levels of toxicity.7,8 It is also known that CA in combination with its structural analogue melamine exerts stronger cytotoxic effects in vitro than CA or melamine alone.9 The oral LD50 in rats is greater than 10,000 mg/kg, while the dermal LD50 in rabbits is greater than 7940 mg/kg.

1,3,5-tris(2-hydroxyethyl) isocyanurate (1,3,5-Tris(2-hydroxyethyl)-1,3,5-triazine-2,4,6(1H,3H,5H)-trione; THIC) (Figure 1, B.) is a derivative of CA that is utilized in the manufacturing of heat-resistant wire enamels, rubber materials, alkyd resins, urethanes, and polyesters, as well as for the synthesis of dyes, agrochemicals, pharmaceuticals, and plasticizers. The sister chromatid exchange assay and the chromosomal aberration test have been used to investigate the toxicity of THIC in vitro on Chinese hamster ovary (CHO) cells. No cytotoxicity or adverse effects were found in these tests.10 There is no information on how THIC affects people or human cells in vitro.

A potent oxidant and chlorinating agent is trichloroisocyanuric acid (1,3,5-trichloro-2,4,6-triazinetrione; TCIC) (Figure 1, C.). Generally used for disinfecting swimming pools, restaurants, and other public areas, it is a bleaching and anti-shrinking agent that is a highly effective broad-spectrum disinfectant with outstanding bactericide and fungal qualities. The compound causes DNA strand breaks and has the strongest cytotoxic effect when compared to two other biocides, according to in vitro studies on TCIC cytotoxicity and genotoxicity towards the fish cell line RTG-2.12 However, there are no reports regarding TCIC toxicity against human cells.

There is little information available regarding the toxicity of CA, TCIC, and THIC to many human cell types, particularly cancer cells, given their extensive industrial use. Therefore, the current work offers data from in vitro cytotoxicity screening employing four human cell lines treated with varying concentrations of CA, TCIC, and THIC in order to shed additional light on the subject. Our findings show that TCIC has the highest cytotoxicity among the three triazines. Remarkably, TCIC has more significant inhibitory effects on cancer cells than on healthy fibroblasts, indicating the possibility of targeted anticancer action..

Figure 1. *Chemical Structures of s-triazine Derivatives.*
(A) cyanuric acid, (B) 1,3,5-tris(2-hydroxyethyl) isocyanurate, (C) trichloroisocyanuric acid.



MATERIALS AND METHODS

Culture Conditions and Cell Lines

A549 (ATCC CCL 185, NBIMCC 2404) originating from lung carcinoma; CaOV (NBIMCC 1108) isolated from testicular cystadenoma; HeLa (ATCC CCL 2, NBIMCC 164) established from cervical adenocarcinoma; and a non-cancerous fibroblast cell line F derived from preputium were the human cell lines used to assess CA, THIC, and TCIC cytotoxicity.

All cell lines were cultivated at 37°C, 5% CO2-atmospheric air mixture, and high humidity in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal calf serum, 100 µg/mL streptomycin, and 100 IU penicillin (all from Merck KGaA, Darmstadt, Germany). In 75 cm2 culture flasks (TPP, Trasadingen, Switzerland), the cells were grown. Trypsinization was used to separate the cells from the culture vessel before the in vitro cytotoxicity tests. The concentration of viable cells was established and set at 100 cells/mL for the clonogenic assays and 1x105 cells/mL for the MTT and Neutral Red (NR) assays.

Assays for In vitro Cytotoxicity

The cytotoxicity of CA, THIC, and TCIC was assessed in vitro using clonogenic, MTT, and NR tests. 96-well plates (TPP, Trasadingen, Switzerland) were seeded with 200 µL of cell suspension per well for the MTT and NR assays. The plates were then cultivated in standard supplemented DMEM for 24 hours at 37°C with 5% CO2/atmospheric air in a humidified incubator. After that, the culture media was swapped out with full DMEM that contained the test substance (such as 1,3,5-tris(2-hydroxyethyl) isocyanurate [C9H15N3O6], cyanuric acid [C3H3N3O3], or trichloroisocyanuric acid [C3Cl3N3O3]) that was acquired from Merck KGaA, Darmstadt, Germany. Three distinct amounts of CA, THIC, and TCIC were measured: 10 µg/mL, 100 µg/mL, and 500 µg/mL. Dulbecco's phosphate buffered saline (DPBS) without calcium and magnesium chloride was used to make stock solutions of the triazines at a concentration of 10 mg/mL (Merck KGaA, Darmstadt, Germany). Standard culture medium was used to dilute the stock solutions in order to get the tested test-concentrations of all triazines. CA, THIC, or TCIC were used to cultivate the cells for 24 and 72 hours, respectively. For every in vitro cytotoxicity test, cyclosporin A (Novartis, Basel, Switzerland) was used as a positive control. The triazines and the substance were tested at the same concentrations. Three duplicates of each sample were plated.

Mosmann has described the basic idea behind the MTT in vitro cytotoxicity assay.13 In the current experiments, all samples were incubated with triazines for 24 and 72 hours before being treated with 5 mg/mL of MTT [3-(4,5-dimethylthiazol-2-yl)-2,4-diphenyltetrazolium bromide supplied by Merck KGaA, Darmstadt, Germany] solution. To reach a final concentration of 0.5 mg/mL, the reagent was diluted directly in the culture medium. The cells were then incubated for three hours at 37°C with 5% CO2 in the air in a humidified incubator. The amount of formazan produced by MTT reduction and accumulated in viable cells was measured at the end of the incubation period. To do this, 100 µL of DMSO was pipetted into each test well after the MTT-containing solution was withdrawn. The cells were shaken at room temperature for 15 minutes before the Synergy-2 reader (BioTek, Winooski, VT, USA) was used to detect absorbance at 570 nm. The percentage inhibition of cell growth rate was calculated using absorbance units from each test sample and from the control cells cultured in standard culture media without triazines. As a result, IC50 values were determined.In accordance with Repetto's instructions, neutral red (NR) uptake tests were conducted.14 HeLa, A549, CaOV, and F cells were grown, separated, seeded on 96-well plates (TPP, Trasadingen, Switzerland), and subjected to triazines in the same manner as for the MTT measurement. The NR assay allows for the assessment of lysosome function and cell culture viability after test-agent treatment.15 In our investigations, cells were stained with NR solution 0.5 mg/mL for 3 hours at 37ºC and high humidity following 24 and 72 hours of triazine exposure. To remove the stain that had developed in the cells, the growth media was then evacuated, and 100 µL of a solution that included 50% ethanol and 1% acetic acid was applied to each sample. The Synergy-2 reader (BioTek, Winooski, VT, USA) was used to measure the absorbance at 540 nm after the culture plates had been gently shaken continuously for 15 minutes. The observed absorbance units were used to quantify the percentage inhibition of cell viability and growth rate. IC50 values were thus calculated.

HeLa, A549, and CaOV single cell suspensions containing 100 cells/mL were seeded on 12-well plates using 0.5 mL/well in order to carry out clonogenic assays16 (TPP, Trasadingen, Switzerland). Triazine solution was introduced to the cells after a 24-hour period. Four distinct doses of the test reagents were used for the assay: 100 µg/mL, 50 µg/mL, 25 µg/mL, and 5 µg/mL. For ten days, the cells were cultivated in DMEM supplemented with triazines until cellular clones were produced. The culture media was then aspirated, and the clones were incubated for 20 minutes with Giemsa dye after being cleaned with Dulbecco's phosphate-buffered saline (DPBS). Following that, colored clones were counted and the stain was removed using distilled water. The following formula was used to determine clonogenic efficiency (CE): CE= (number of clones in the sample)/(number of clones in the control)*100. The percentage inhibition of clonogenic efficiency was computed using this value: % inhibition=100-CE.

Intracellular ATP Concentration Measurement

HeLa, A549, and F cells at a concentration of 0.5x106 cells/well were seeded on 6-well plates (TPP, Trasadingen, Switzerland) and incubated for 24 hours under standard conditions in order to measure intracellular ATP. The cells were then treated for 24 hours to 100 µg/mL TCIC. The cells were then separated, lysed, and cleaned with sterile DPBS. Using a standard ATP Determination Kit (A22066, Molecular Probes, Life Technologies, USA), the intracellular ATP concentration was determined in accordance with the manufacturer's instructions. The luciferase enzyme catalyzed a bioluminescent reaction between D-luciferin and ATP, which served as the basis for the assay.17

Cellular Morphology Analysis

A typical inverted light microscope, the Inverso (Medline Scientific, Chalgrove, Oxon, UK), with software and a high resolution digital camera, the Si-3000, was used to do the morphological analysis.

Data

Using StatView software (SAS Institute, USA), the non-parametric Mann-Whitney U-test was used to assess the results' statistical significance. P values that were calculated and less than 0.05 were deemed statistically significant.

RESULTS

MTT and NR Assays for the Measurement of CA, THIC, and TCIC's In vitro Cytotoxicity

The current investigation used four human cell lines: a finite cell line made from normal dermal fibroblasts and three cancer cell lines obtained from patients with various neoplastic tumors. Evaluation of both possible anticancer efficacy and general cytotoxic effects is made possible by this panel of several cell types. Figures 2 and 3 display the outcomes of the MTT and NR in vitro tests, respectively. Cyclosporine A, an immunosuppressive and antitumor drug, was used as a positive control in these studies and had a potent cytotoxic impact. As an untreated control, cells were cultivated in conventional growth media for the same amount of time. Data from untreated and test agent-exposed cells were used to assess the percentage inhibition of cell growth and development. IC50 values were calculated for a 72-hour exposure period (Table 1).

The results of the MTT experiment show that the cells' reactions to CA, THIC, and TCIC treatment are both dose-dependent and time-dependent. While normal fibroblasts showed a very low level of inhibition (on average 20%, 5%, and 3% for 24-hour exposure to CA, TCIC, and THIC, respectively) that did not increase with exposition time, it is interesting to note that the percentage inhibition of cancer cell growth increased with longer exposure to triazines (Figure 2, B., D., F.). Furthermore, the NR assay findings demonstrated the exact opposite trend: inhibition of fibroblast culture development was generally detected after CA and TCIC administration, with the inhibitory effects of CA and TCIC decreasing fivefold and tenfold during the longer exposition test duration (Figure 3, G., H.). Only the NR assay was able to detect these effects (Figure 3, G.), indicating toxicity unique to lysosomes. The cells were able to overcome the adverse effect, nevertheless, as evidenced by their decreased % inhibition following a prolonged exposure to the triazines (72 hours) (Figure 3, H.). However, the

Table 1. *IC_{50}(μg/ml) According to Different in vitro Cytotoxicity Assays*

Cell lines	Test-compounds	IC_{50} MTT assay*	IC_{50} NR assay*	IC_{50} Clonogenicity
A549	CA	-	-	-
	TCIC	151.95 (±5.62)	-	7.95 (±1.24)
	THIC	-	-	-
	CSP	57.5 (±7.35)	45.57 (±1.43)	7.5
HeLa	CA	383.64 (±3.21)	447.37 (±3.25)	-
	TCIC	80.01 (±2.65)	-	9.57 (±1.14)
	THIC	-	-	-
	CSP	56.7 (±3.57)	51.31 (±5.87)	7.5
CaOV	CA	-	-	7.93 (±0.35)
	TCIC	326.13 (±7.35)	-	-
	THIC	-	-	-
	CSP	37.86 (±3.89)	64.53 (±10.59)	7.5
Fibroblasts	CA	-	-	n.d.
	TCIC	-	-	n.d.
	THIC	-	-	n.d.
	CSP	54.7 (±6.35)	89.2 (±7.32)	n.d.

*The values for MTT and NR assays are calculated using data from 72-hours exposition to triazines.
n.d. – not detected; CA – cyanuric acid; TCIC – trichloroisocyanuric acid; THIC – 1,3,5-tris (2-hydroxyethyl) isocyanurate; CSP - cyclosporine

Figure 2. *Inhibitory Effects of Cyanuric Acid, Trichloroisocyanuric Acid and 1,3,5-Tris(2-Hydroxyethyl) Isocyanurate Measured by MTT Assay.*
(A), (C), (E), (G) A549, HeLa, CaOV cells and fibroblasts were cultured in growth medium containing triazines for 24 h and analyzed by MTT assay; (B), (D), (F), (H) A549, HeLa, CaOV cells and fibroblasts exposed to triazines for 72 h. The results are shown as mean ± SE. CSP-cyclosporine A.

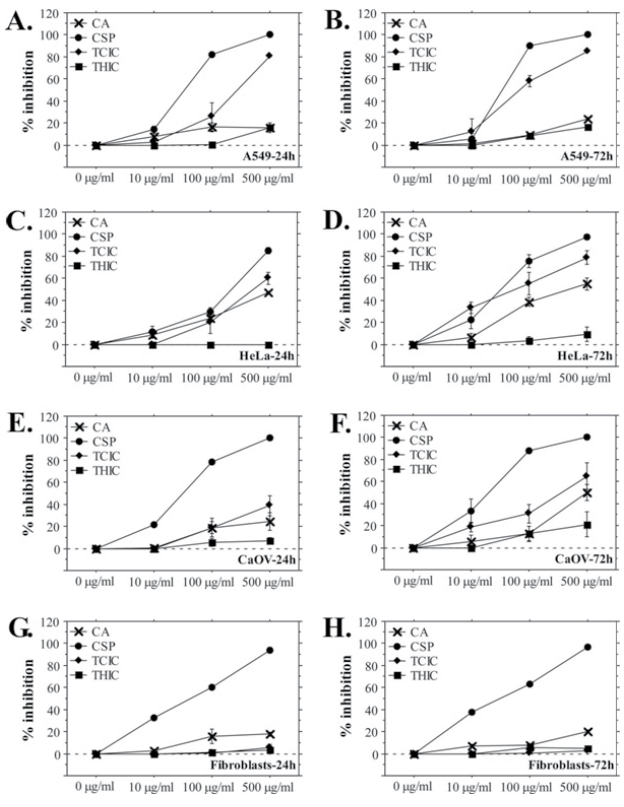
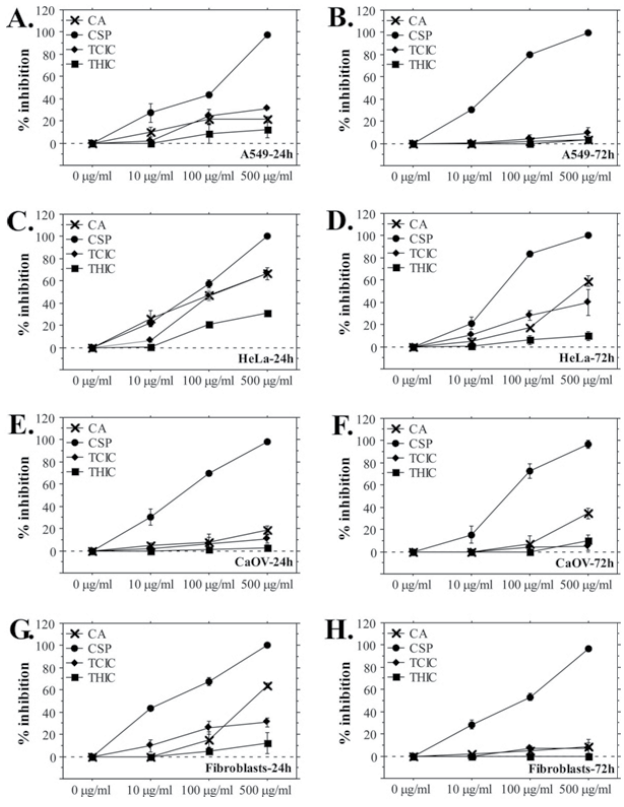


Figure 3. *Neutral Red Assays with A549, HeLa, CaOV and Fibroblast Cells Exposed to s-triazines.*
(A), (C), (E), (G) A549, HeLa, CaOV cells and fibroblasts treated with triazines for 24 h; (B), (D), (F), (H) A549, HeLa, CaOV cells and fibroblasts after 72-hours exposure to triazines. The results are present as mean ±SE. CSP – cyclosporine A.

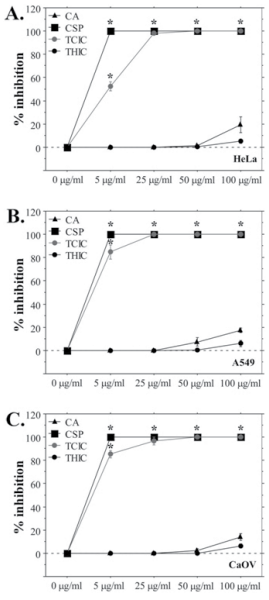


MTT assay demonstrated that triazines had more potent inhibitory effects on cancer cell types. In fact, HeLa was the most sensitive cell line to triazine treatment, and only NR assays with HeLa cells demonstrated a significant percent inhibition (up to 45% for 72-hour exposure to TCIC, 65% after 72-hour treatment with CA, and 10% for 72-hour exposure to THIC) that was comparable to the data from MTT assays (Figure 2, B., C., D., F.). The most cytotoxic triazine, TCIC, is clearly identified by comparing the inhibitory effects of the three test chemicals. Its IC50(72) values fall between 80 and 330 µg/mL (80.01 µg/mL for HeLa, 151.95 µg/mL for A549, and 326.13 µg/mL for CaOV). The order of cytotoxicity rating is TCIC>CA>THIC. Cellular growth and vitality were not significantly inhibited by THIC. Cyclosporine A exhibited a greater inhibitory impact than any of the triazines.

TCIC Prevents Cancer Cell Lines from Being Clonogenic

The cytotoxicity of CA, THIC, and TCIC was examined using clonogenic assays in addition to NR and MTT assays. The purpose of these tests was to ascertain whether exposure to the triazines had an impact on the cells' capacity for proliferation, which is correlated with their clonogenic efficiency. Because normal fibroblasts have a relatively poor capacity for clonogenic activity, these experiments were limited to cancer cell lines. Figure 4 illustrates how TCIC significantly reduced the clonogenic efficiency of every cell line, which was comparable to the impact of cyclosporine A. At the highest tested triazine doses, CA and THIC showed noticeably less inhibition, ranging from 5 to 20%. The toxicity of TCIC (7–10 µg/mL IC50) differs significantly from that of the other two studied triazines (CA and THIC, with IC50 >100 µg/mL), according to calculated IC50 values based on the clonogenicity data (Table 1). These findings support the findings of the MTT and NR assays

Figure 4. *Clonogenicity Inhibition of Cancer Cell Lines Induced by Cyanuric Acid and Its Derivatives.*
*Clonogenic assay results for HeLa cell line; (B) A549 clonogenicity inhibition following exposure to triazines or cyclosporine; (C) Inhibition of clonogenic efficiency of CaOV cells after exposure to CA, THI, TCIC and CSP. The data are present as mean ±SE. *p<0.05.*

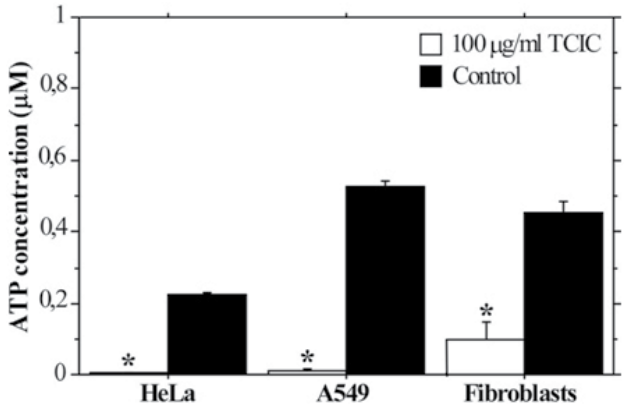


and show that TCIC has a potent cytotoxic effect on the level of proliferative capacity, which is similar to the impact of well-known cytostatic drugs like cyclosporine.

Exposure to TCIC Affects Intracellular ATP Concentration

Out of the three triazines that were evaluated, TCIC showed the highest level of cytotoxicity. Therefore, we assessed the ATP levels in HeLa, A549, and F cell lines to learn more about its effects on cellular physiology. For 24 hours, the cells were treated to 100 µg/mL TCIC. The notable discrepancy between the data from the clonogenic assays and the MTT/NR experiments led to the selection of this dose. IC50 values based on clonogenicity assays were more than ten times lower than those based on MTT and NR data, which were often higher than 100 µg/mL. During the same test time, the intracellular ATP levels of cells treated with TCIC were contrasted with those of control HeLa, A549 cells, and fibroblasts cultured in regular culture media (Figure 5). All cell types exposed to TCIC showed a significant ($p<0.05$) drop in ATP levels. The ATP levels of fibroblasts treated with TCIC were marginally great

Figure 5. *Intracellular ATP Concentrations in Control Cells and Cells Treated with 100 µg/ml TCIC.*
*The cells were cultured in growth medium containing TCIC for 24h. The graph represent mean±SE. *p<0.05*

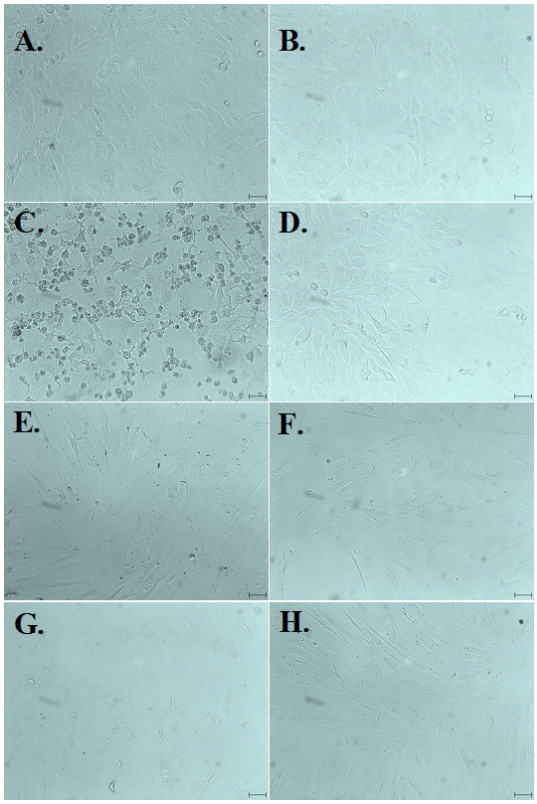


er than those of cancer cells treated with TCIC. The NR and MTT assay results, which indicated less inhibition in normal fibroblasts, are supported by this tendency.

In vitro, TCIC Affects Cellular Morphology

Triazine-treated Microscopical analysis of triazine-treated cells was performed to assess the effects of CA, TCIC, and THIC on cellular morphology. Figure 6 displays images of A549 and F cells. Microscopic inspection revealed a significant impact on the morphology of cells in culture following exposure to TCIC, which was consistent with the findings from MTT, NR, and clonogenic assays. Most of the cells shrunk and displayed aberrant morphology as a result of the disruption of the cell monolayers. When 100 µg/

Figure 6. *Effects on Cellular Morphology Observed Following Treatment with CA, THIC and TCIC.*
A549 cells and fibroblasts were treated with 100 µg/ml triazines for 24 hand then analyzed microscopically. The photos were taken during observation with 25x objective yielding 250 magnification. The black line on each photo indicates 10□m. (A), (B), (C) - A549 cells exposed to CA, THIC, TCIC respectively; (D) A549 control; (E), (F), (G) show fibroblasts treated with CA, THIC and TCIC accordingly; (H) control F culture.



mL TCIC was added to the cells, this became clear. The significant effect was not produced at lower triazine concentrations. However, the integrity of cell culture monolayers and cellular morphology were unaffected by CA and THIC treatment.

DISCUSSION

CA, THIC, and TCIC are members of the 1,3,5-triazine (s-triazine) group, which is one of the earliest groups of organic compounds with distinct chemical characteristics. Since it can participate in a variety of interactions, including coordination, hydrogen bonds, electrostatic and charge-transfer attractions, and aromatic-stacking interactions, the s-triazine structure is regarded as an exceptional tool in organic synthesis.18 The symmetric structure of 1,3,5-triazines and their high affinity for binding to numerous enzymes make them a suitable candidate for the production of compounds with therapeutic activity and various applications. Many s-triazine derivatives are utilized extensively as chemotherapeutics, herbicides, insecticides, antibacterial agents, corrosion inhibitors, enzyme inhibitors, etc. because of their special pharmacologic characteristics. Among the many uses for this class of triazines are cyanuric acid, trichloroisocyanuric acid, and 1,3,5-tris(2-hydroxyethyl) isocyanurate. There is little information available regarding their toxicity to human cells. This fact served as the impetus for the current study, which offers fresh insights into this area. Here, we show that CA and THIC show modest toxicity, while TCIC has a high amount of cytotoxicity, with an IC50(72) in the 80–151.95 µg/mL range. Out of the three triazines that were examined, THIC seems to be the least harmful substance. Our findings support earlier reports of low cytotoxicity CA and THIC in non-human cell lines (6, 8–10). Sanchez-Fortun et al. have examined the cytotoxicity and genotoxicity of TCIC in relation to two additional disinfection agents, sodium bromide and Oxone®. When administered to the fish cell line RTG-2.12, they claim that TCIC causes DNA strand breaks at 1.2 mg/L.12 Their findings show that fish cells are more sensitive than other biological material evaluated. In contrast to the current study, RTG-2 cells once again exhibit higher sensitivity to TCIC, with an IC50 of 30.73 µg/mL (48) determined by NR assay data.12 We were able to determine the TCIC IC50 for human cell lines based on MTT assay results, while the NR assays suggested higher than 500 µg/mL IC50 (72). For instance, the Ames test with Salmonella cells demonstrated that TCCA does not induce gene mutations.

Human embryonic kidney cells (HEK-293), Madin-Darby canine kidney cells, Crandell feline kidney cells, rat NRK-52E, and human HEK293T cells have all been used to study the toxicity of cyanuric acid and mixtures of CA and melamine.7 The reported data show low CA cytotoxicity with IC50 values greater than 1 mg/mL.7 This is consistent with our findings.

There have been no reports of THIC's toxicity to human cells assessed in vitro. Salmonella typhimurium TA97, TA98, TA100, TA1535, and TA1537 were used in a study that showed no cytotoxicity or adverse effects of THIC at concentrations of 100–10,000 µg/plate, both with and without activation.20 Using Chinese hamster ovary cells, sister chromatid exchange assays showed no genotoxic effect at THIC concentrations of 0.402, 1.210, 4.020 µg/mL without activation and 0.381, 1.140, 2.290, and 3.810 µg/mL with activation.10 Our results show very weak cytotoxicity of THIC against human cancer cell lines and no long-term toxicity to normal dermal fibroblasts, thus generally corroborated the findings on other cell types.Compared to other non-triazine heterocyclic compounds, the s-triazine ring offers a convenient foundation for the development of biologically active compounds with lower toxicity and higher efficiency.21 While few compounds have advanced to the clinical trial stage thus far, the prospect of discovering safe agents with therapeutic potential—particularly in the treatment of cancer—inspires researchers to look for a suitable compound within the s-triazine derivatives group. Research in this area has so far concentrated on compounds with complicated structures and high molecular weights. Simple s-triazine derivatives are useful industrial tools with a variety of uses, including the manufacture of antitumor drugs, but their possible anticancer effects are unknown. In order to analyze the possible anticancer effect of the investigated triazines, we included both cancer and normal cell lines in our in vitro cytotoxicity tests. Our findings do, in fact, support TCIC's selective anticancer action. In cancer cells, this triazine suppressed mitochondrial function. Treatment with TCIC did not significantly affect normal fibroblasts. There was also a significant impact on the clonogenic efficiency of cancer cells.

There are no published detailed structure-activity relationship (SAR) investigations of CA, THIC, or TCIC. Nonetheless, it is evident that these chemicals' cytotoxicity is highly influenced by their chemical makeup and structure. The symmetric distribution of nitrogen atoms in the s-triazine ring makes it easier for various functional groups to bind to positions 2, 4, and 6.2 The reactivity of various s-triazines is determined by the characteristics of each substituent as well as the unique features of the ring structure. Up to three chlorine atoms can be bound by CA to produce TCIC. According to certain theories, TCIC may work as an in situ source of chlorine ions, which catalyze various processes involving organic transformations. It is mostly utilized as an oxidation and chlorination agent.11 These actions have the potential to alter and change a variety of cellular molecules in vitro, upsetting normal cell architecture and physiology. Thus, TCIC's chemical structure and characteristics may be responsible for its notable cytotoxic effects; nevertheless, more research is required to elucidate the toxicity mechanisms.

Various techniques have been created and used to assess cytotoxic effects in vitro. For instance, the NR test, the LDH assay, the assay based on the reduction of tetrazolium salts, the assay for figuring out the number of viable cells and the amount of total protein or nucleic acid, etc. A comparison of some of the most widely used in vitro cytotoxicity tests revealed that MTT and NR tests have the best sensitivity.15 This explained why NR and MTT assays are employed to evaluate the in vitro cytotoxic effects of CA, THIC, and TCIC. These assays enable the discrimination of general cytotoxic effects, organelle-specific toxicity, and anticancer potential when used on a panel of human cell lines, including normal and cancer cells. However, a variety of in vitro cytotoxicity assays evaluating various cellular characteristics and activities must be carried out in order to provide a trustworthy evaluation of these effects. Therefore, to examine the effect of triazine therapy on the potential for cell proliferation, we performed clonogenic experiments in addition to MTT and NR assays. Although clonogenicity assessments were first intended to gauge how radiation affected cells in culture, they are now frequently employed to assess the cytotoxic and antitumor potential of various test agents. The proliferative capacity of cells that have been inhibited by the test substance can be evaluated using these assays.The lack of proliferative ability is the primary cause of cell death. Accordingly, clonogenic cells are those that maintain their capacity to proliferate endlessly and produce a unique clone. Reproductively dead cells are those that maintain metabolic activity, divide once or twice, but are unable to create a clone.16 The ability of a cell to develop and divide in order to create a visually detectable clone demonstrates its intact proliferative potential. The cells in the clonogenic experiments conducted in this study were exposed to a specific concentration of the test agent for ten days until clones developed in either the control or the test samples. As a result, these tests allow evaluation of the long-term inhibitory effects of triazines and demonstrate the potent cytotoxicity of TCIC in conjunction with the noticeably less severe adverse effects of prolonged exposure to CA and THIC. These findings are supported by ATP assays, which show that intracellular ATP is always greatly decreased by TCIC exposure, which results in proliferative incapacity. These findings are corroborated by MTT and NR data, which indicate that TCIC primarily impacts mitochondrial function, which is linked to ATP synthesis.Furthermore, the current study demonstrates the necessity of using a variety of in vitro cytotoxicity tests to more accurately describe the inhibitory effects of test agent exposure. Thus, we were able to ascertain TCIC's toxicity specific to mitochondria. Additionally, cell type-specific inhibition can be identified through toxicity testing on a panel of cell lines. Here, we demonstrate the selective anticancer toxicity of TCIC in addition to its potent cytotoxic effects, with the HeLa cell line exhibiting the highest sensitivity.

CONCLUSION

The cytotoxicity of s-triazine derivatives with a broad range of industrial applications is discussed in the current work. We show that two of the studied compounds, CA and THIC, are not very harmful to human cells, whereas TCIC had a significant impact on cellular morphology, mitochondrial functions, and reproductive capacity. Trichloroisocyanuric acid > Cyanuric acid > 1,3,5-tris(2-hydroxyethyl) isocyanurate is the order of test-compound cytotoxicity in vitro. Trichloroisocyanuric acid exhibits a selective antitumor action because it is more harmful to cancer cells than to healthy fibroblasts.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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