# Cytotoxicity of Dinitrotoluenes (2,4-DNT, 2,6-DNT ) to MCF-7 and MRC-5 Cells

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**Abstract:** DNTs are considered possibly carcinogenic to humans (Group 2B) because there is inadequate evidence in humans for carcinogenicity though there is sufficient evidence in experimental animals. In this study, MCF-7 (breast) and MRC-5 (lung) cells were exposed to a serial dilution of 2,4 and 2,6 DNTs (control, 1-500 ppm) in 96 well tissue culture plates. After various time intervals (24, 48, 72 and 96 hrs) the plates were washed, and 100μl fluorescein diacetate solution (10 μg/ml in PBS) was added column wise to each well, and incubated at 37°C for 30 - 60 min before reading the fluorescence with a spectrofluorometer at excitation and emission wavelengths of 485 and 538 nm respectively. Spectrofluorometeric readings were converted to percentages of cell survival. Regression analysis was conducted to determine the relationship between cell survival and exposed concentration. Linear equations derived from the regression analysis were used to calculate the $LC_{50}$ values. Results indicated that 2,6 DNT was more toxic to breast cells; $LC_{50}$ values were 445 and 292 ppm at 24 and 48 hours respectively compared to 2,4 DNT showing $LC_{50}$ values of 570 and 407 ppm at 24 and 48 hours, respectively. No significant differences in toxicity existed between the two chemicals with regard to lung cells. Contrary to the above observation, 2,4 DNT was more toxic to breast cells; $LC_{50}$ values were 407 and 238 ppm at 24 and 48 hours respectively compared to lung cells showing $LC_{50}$ values of 527 and 402 ppm at 24 and 48 hours respectively. No significant difference existed for 2,6 DNT between the two cell lines. Lungs cells were more resistant to the two chemicals.

**Keywords:** Dinitrotoluenes, cytotoxicity, breast cancer cells (MCF-7), lung cells (MRC-5).

## Introduction

DNTs (2,4 and 2,6) are produced through dinitration of toluene with nitric acid in the presence of concentrated sulfuric acid [1]. They are used in munitions as smokeless propellant powders, as gelatinizing and plasticizing agents in commercial and military explosive compositions and in the manufacture of dyes [2]. During the production of Trinitrotoulene (TNT) small amounts of DNT isomers also occur as byproducts [3; 4]. Leaching of wastewaters from disposal sites that contain significant amount of DNT arising from their preparation and production at Army ammunition plants have been identified in surface water, groundwater and in soils [5].

Major exposure routes of DNTs (via ingestion, inhalation, and dermal contact) have been associated with a significant number of health effects [6]. Exposure to nitroaromatic compounds intially may result in mild irritation of respiratory passages producing nasal discomfort, sneezing, epistaxis, and rhinitis, as well as irritation of the skin producing erythema and papular eruptions progressing to desquamation and exfoliation [1]. Absorption of sufficient amounts of DNTs through the skin or lungs has been published else where in literature. These exposure routes may produce signs of cyanosis due to methemoglobin formation, toxic jaundice, and hepatitis due to severe liver damage, aplastic anemia due to damage to the erythropoietic system, eye damage (cataract), menstrual disorders (hypo- or hypermenorrhea), neurological manifestations (neurasthenia, nystagmus, and irregularities in tendon reflexes), and kidney damage [3; 6, 7]. On the average 500 workers in the USA are exposed to DNTs when they are used as intermediates in the production of munitions and explosives [1].

DNTs are possibly carcinogenic to humans because there is inadequate evidence in humans for carcinogenicity though there is sufficient evidence in experimental animals [8]. It has been reported that, chronic exposure to 2,4-DNT induces significant number of cancers in laboratory animals including adenoma, fibroma, hepatocellular carcinoma, and mammary tumors in rats [9]. In the reversion assays with *Salmonella typhimurium*, with or without metabolic activation, TNT was found to be strongly mutagenic, while 2,4-DNT and 2,6-DNT were weakly mutagenic. [10]. Based on these laboratory and other studies, the U.S. Environmental Protection Agency considers DNTs as probable human carcinogens-Group B2 [11].

In this work we exposed DNTs to breast and lung cells to determine the cytotoxic effects of DNTs. These cells were chosen due to their differences in morphology, age, ploidy and characteristics [12].

## Materials and Methods

MCF-7 (breast) and MRC-5 (lung) cells were grown with DMEM supplemented with 10% fetal bovine serum (FBS) and 1% streptomycin and penicillin at 37°C in a 5%$CO_2$ incubator to 90-100% confluence. The old medium was removed and cells washed with phosphate buffer saline (PBS), trypsinized with about 4 mL of 0.25% (w/v) trypsin-0.03% w/v) EDTA. Fresh medium was added and centrifuged in 50 mL conical tubes at 3000 RPM for 10 minutes. Cells were incubated for 24, 48, 72 and 96 hours at $\pm$ 37°C in 5% $CO_2$. Plates were washed once with 200μl PBS/well. Hundred micro-liters (μl) of Fluorescien Diacetate solution (10 μg/ml in PBS) was added columnwise to each well and incubated at 37°C for 30 - 60 minutes before reading the fluorescence with a Spectrofluorometer at excitation and emission wavelength of 485 and 538 nm respectively. Spectrofluorometer readings were converted to cell survival. Regression analysis was conducted between cell survival and exposed concentrations. Linear equations derived from the regression analysis were used to calculate the LC 50's.

## Results

Figure 1 is a regression analysis conducted on the breast cells exposed to different concentrations of 2, 4 DNT. The graph shows an inverse correlation between 2, 4 DNT concentration and percent cell survival. It was observed that below 100ppm of 2, 4 DNT exposure, almost all the cells survived and at 500ppm of 2,4 DNT exposure almost 100 % cell death occurred. Similar observation were made when the breast cells were exposed to 2,6 DNT. With the lung cells exposure to both 2,4 and 2,6 DNT, the regression analysis yielded comparable results to the ones obtained for the breast cells.

Figure 2 depicts the lethal concentration ($LC_{50}$) for breast cells exposed to DNT (2,4 and 2,6). The figure shows the $LC_{50}$ were time dependent. The highest $LC_{50}$ was observed at 96 hours (138.4 ppm for 2,4 DNT and 121.1 ppm for 2,6 DNT) and the lowest at 24 hours (569.9 ppm for 2,4 DNT and 445.3 ppm for 2,6 DNT ).
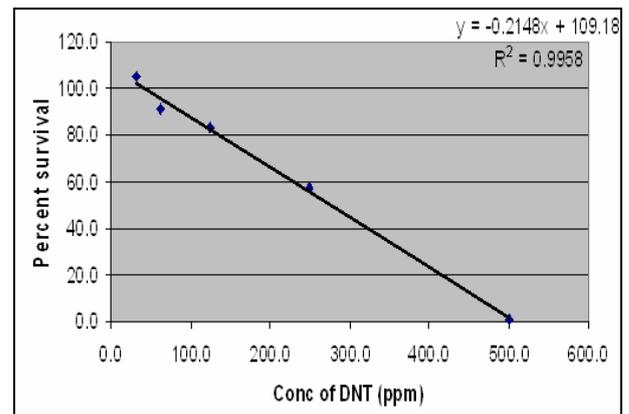


**Figure 1:** Regression analysis for breast cells exposed to different concentrations of 2,4 DNT.
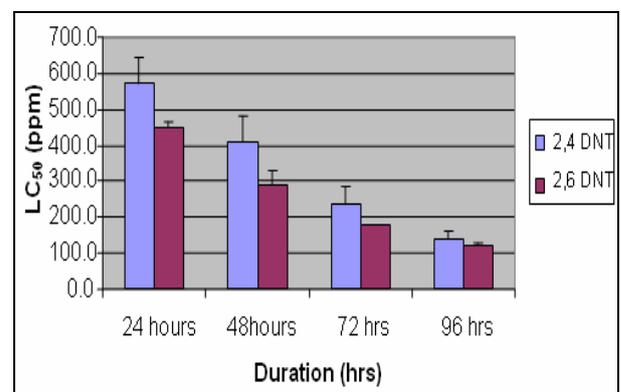


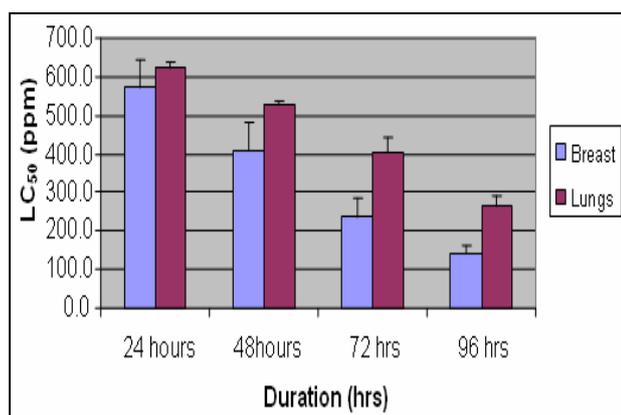**Figure 2:** $LC_{50}$ values of 2,4 and 2,6 DNT for breast cells exposed at various times.

Figure 3 compares the $LC_{50}$ for lung cells when exposed to 2,4 DNT and 2,6 DNT. At 24 hours the $LC_{50}$ were 622 and 663 ppm for 2,4 DNT and 2,6 DNT respectively. At 96 hours, the $LC_{50}$ were 265 and 192 ppm for 2,4 DNT and 2,6 DNT.
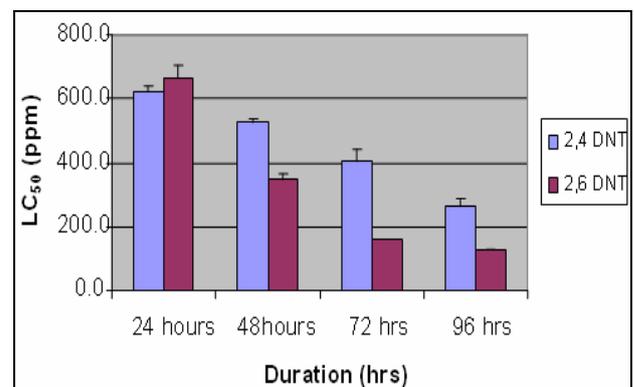


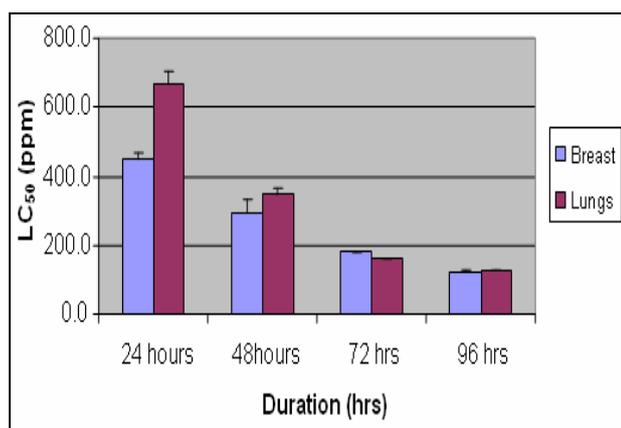**Figure 3:** $LC_{50}$ values of 2,4 and 2,6 DNT for lung cells exposed at various times.

The $LC_{50}$ for both lung and breast cells were compared to see if there was any difference between the two cells when exposed to 2,4 DNT. Figure 4 shows that there was a significant difference between lung and

breast cells (p>0.05): 2,4 DNT was more toxic to breast cells than to lung cells.



**Figure 4:** $LC_{50}$ values of 2,4 DNT for breast and lung cells exposed at various times.

Similar comparison was made between lung and breast cells when exposed to 2,6 DNT (figure 5). Over all the result yielded no significant difference between the two cells though the $LC_{50}$ at 24 hours was higher for breast cells than for lung cells.



**Figure 5:** $LC_{50}$ values of 2,6 DNT for breast and lung cells exposed at various times.

**Discussion**

It has been reported by Tchounwou *et al* [13] that 2,4-DNT was more toxic than 2,6-DNT when exposed to Hep G2 cells. This observation was contrary to what was observed in this study. We observed in this study that 2,6 DNT was more toxic to breast cells compared to 2,4 DNT and with the lung cells no significant differences in toxicity existed between the two chemicals. The possible explanation between these results might be due to the apparent biological differences among the cells. We also observed that 2,4 DNT was more toxic to breast cells than to lung cells and no significant difference existed for 2,6 DNT between the two cells in this study. In summary, lungs cells were more resistant to the two chemicals. Again, the possible explanation for the observed differences between lungs and breast cells might be due to the apparent biological differences between the two cells.

There were major differences between the two cells in terms of morphology, age, ploidy and characteristics. The lung cell is a fibroblast, embryonic and diploid while the breast cell is epithelial, from adult donor and aneuploid. The breast cell is estrogen receptor positive while the lung cell is not. The breast cell is continuous and from neoplastic tissue while the lung cell is finite and from normal tissue [12].

Much of the acute toxicity test with DNT had been done on rodents. It has been reported by Vernot *et al.* [14] that the $LD_{50}$ for 2,6-DNT and 2,4-DNT were 1,000mg/kg and 1,630mg/kg, respectively for CF-1 mice. They also reported that the $LD_{50}$ for male Sprague Dawley rats for 2,6-DNT and 2,4-DNT as 180 mg/kg and 270 mg/kg, respectively.

Levine *et al*. [15], reported the $LD_{50}$ for 2,4-DNT exposures to male and female Swiss mice, as 1,954 mg/kg and 1,340 mg/kg, respectively. They also reported the $LD_{50}$ for male and female CD rats exposed to 2,4-DNT as 568 mg/kg and 650 mg/kg, respectively. With 2,6-DNT, the $LD_{50}$ for male and female Swiss mice were 621 mg/kg and 807 mg/kg respectively; for male and female CD rats the $LD_{50}$ were 535 mg/kg and 795mg/kg respectively. From the above published results, 2,4 DNT was more toxic than 2,6 DNT.

**Conclusions**

Our results indicated that 2,6 DNT was more toxic to breast cells compared to 2,4 DNT. $LC_{50}$ values were 445 and 292 ppm at 24 and 48 hours respectively for 2,6 DNT, and 570 and 407 ppm at 24 and 48 hours respectively for 2,4 DNT. No significant differences in toxicity existed between the two chemicals with regard to lung cells. Contrary to the above observation, 2,4 DNT was more toxic to breast cells; $LC_{50}$ values were 407 and 238 ppm at 24 and 48 hours respectively compared to lung cells showing $LC_{50}$ values of 527 and 402 ppm at 24 and 48 hours respectively. No significant difference existed for 2,6 DNT between the two cell lines. Lung cells were more resistant to the two chemicals. The observed toxicity differences between the lungs and breast cells were contrary to our expectation since it was expected that the breast cells would be more resilient to the chemicals than the lung cells.

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