A NOVEL APPROCH TO EXTRAPOLATE EPIAIRWAY IN VITRO INHALATION TOXICITY DATA TO IN VIVO INHALATION TOXICITY ASSESSMENT

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ABSTRACT

Background and Purpose: In vitro inhalation toxicity was assessed to evaluate the potential respiratory toxicity of two test articles of the same organotin chemical class, a known industrial chemical Dimethyltin dichloride (DMTC) and a new organotin chemical of the same class (MBT-1).

Methods: Two in vitro experiments were conducted using reconstructed 3D human airway epithelial tissues (EpiAirway AIR-100, MatTek EpiAirway™). The first experiment tested both chemicals dissolved in vehicle at concentrations recommended by the MatTeck protocol (20–200 mg/mL) to determine each chemical's GSH (Globally Harmonized System Classification hazard category, 2003). Then extrapolation of the expected in vivo air concentration was made based on the GSH classification levels of in vivo toxicity. To assess the relevance of established inhalation toxicity to a real-world exposure scenarios, a second experiment was performed using significantly lower in vitro concentrations—0.002 – 0.02 mg/mL at serial dilutions— representing a 1,000- to 10,000-fold reduction from the toxic in vitro doses used in the first Experiment. These concentrations were chosen based on the worst-case predicted exposure levels in the human breathing zone after the possible spillage of these chemicals.

Results: Both chemicals showed significant cytotoxicity in the first experiment at the tested in vitro concentrations, with the cell viability falling well below the 75% threshold, classifying them within to GSH Hazard Category 1 or 2. Based on the known acute in vivo toxicity of DMTC, this experiment correctly placed this chemical in the GSH classification.
No cytotoxic effects were observed at any of in vitro lower concentrations, as all cell viability measurements remained above 75% relative to vehicle controls.

Conclusions: These findings indicate that, despite high toxicity of the tested chemicals, the levels of DMTC and MBT-1 expected in occupational settings (similar to what people might be exposed to at work) do not pose a significant risk to human respiratory health. A novel approach to correlate in vitro concentration range (in concentrations preprepared in liquid vehicle) to real in vivo air concentration in the working breathing zone was used in this study. Additional studies with the air pollutant of other GHS categories are needed to confirm a validity of this approach.

OBJECTIVES

The main goal of this study was to assess *in vitro* inhalation toxicity of two test articles of the same organotin chemical class, a known industrial chemical Dimethyltin dichloride (DMTC) and a new organotin chemical of the same class (MBT-1) and extrapolate an *in vitro* EpiAirway data to *in vivo* inhalation toxicity assessment in the real occupational industry conditions.

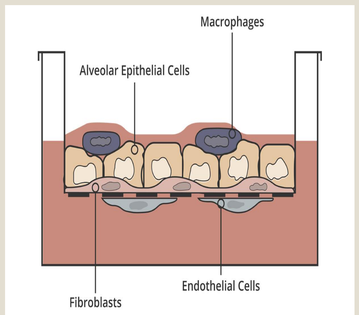
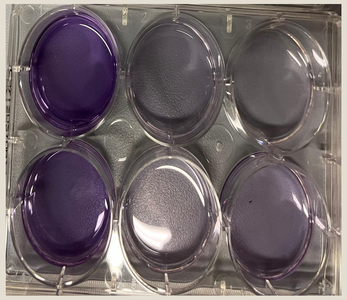


Figure 1. Schematic diagram of EpiAlveolar tetra-culture system containing primary endothelial cells, fibroblasts, epithelial cells and optional macrophages.

Figure 2. Example of the MTT after extraction on the 6-well plate in Experiment 1.

Oil control | Positive control | MBT-1



MATERIALS AND METHIODS

This study was conducted using MatTek's EpiAirway™ AIR-100 human 3D tissue model, following their standardized toxicity testing protocol (MK-24- 007-0072). Two experiments were performed to evaluate the respiratory toxicity of a known highly toxic organotin industrial chemical Dimethyltin dichloride (DMTC) and a new organotin of the same chemical class, MBT-1: one using high concentrations to assess acute effects and hazardous class, and another using much lower concentrations to reflect realistic exposure scenarios.

MatTek's EpiAlveolar system consists of normal, human-derived alveolar epithelial cells co-cultured with donor-matched fibroblasts and human microvascular endothelial cells on specially prepared, optically clear cell culture inserts Human-derived macrophages can also be incorporated into the three-dimensional, highly differentiated model (Figure 1).

The MatTek EpiAirway™ tissues were cultured on specially prepared cell culture inserts and were shipped to CBT as kits, containing 24 tissues on shipping agarose together with the necessary amount of culture media, DPBS, 6-well plates, and 24-well plates. In addition, the MTT kit (containing MTT concentrate, diluent, and extractant) was provided by MatTek.

Upon arrival, tissues were equilibrated overnight in assay medium at 37°C with 5% CO_2 and high humidity. The following day, tissues were rinsed with TEER buffer to remove mucus before dosing. A 100 µL dose of each test solution at appropriate concentration was applied to the surface of the tissues, followed by a 4-hour incubation. After exposure, tissues were rinsed three times with TEER buffer and transferred to fresh medium for an additional 20- hour incubation.

Experiment 1 (High Dose): DMTC and MBT-1 were tested at concentrations of 20, 100, and 200 mg/mL recommended by TaTek's protocol. Toluene (200 mg/mL) was used as the positive control, deionized pure water was used as a negative control and a solvent for DMTC and corn oil served as both the negative control and solvent for MBT-1 (as it is not soluble in water).

Experiment 2 (Low Dose): The same test articles were evaluated at concentrations of 0.002, 0.01, and 0.02 mg/mL to model potential workplace exposure levels. The same controls/solvent were used as in experiment 1.

Following the 24-hour protocol, duplicate tissues were used for each test condition. Both tissues were processed using the MTT assay to measure cell viability (Example is presented in Figure 2). Absorbance readings were obtained using a SpectraMax M4 microplate reader, and relative viability was calculated as a percent of the negative control. The ED-25 (effective dose reducing viability by 25%) was calculated using the spreadsheet tool provided by MatTek.

Figure 3A: Experiment 1. DMTC

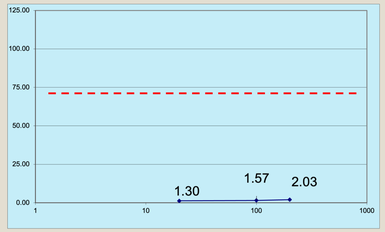


Figure 3B: Experiment 1. MBT-1

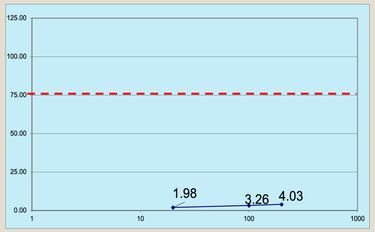


Figure 4A: Experiment 2. DMTC

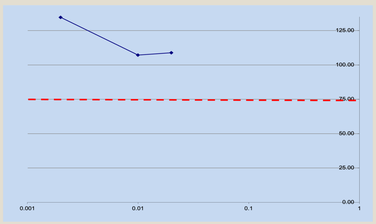
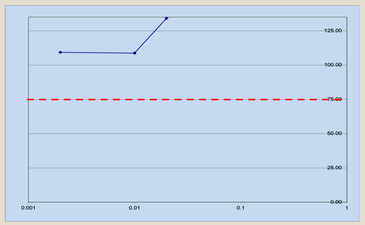


Figure 4B: Experiment 2. MBT-1



RESULTS

Experiment 1: Both DMTC and MBT-1 were tested at 20, 100, and 200 mg/mL to assess acute cytotoxicity on EpiAirway™ human airway tissues. Results showed strong and consistent cytotoxicity for both compounds across all doses, with tissue viability levels ≤4% in every case (decrease of 96% or more). The calculated ED-25 values for both chemicals were at or below 20.0 mg/mL, indicating high toxicity even at the lowest dose tested. These findings are summarized in Table 1 and Figure 3.
Experiment 2: DMTC and MBT-1 were tested at low concentrations (0.002, 0.01, and 0.02 mg/mL) to simulate realistic workplace exposure levels. Both compounds showed no cytotoxicity, with all tissue viability values well above 75%, and ED-25 values reported at the concentration greater than 0.02 mg/mL. These results indicate that while both chemicals were highly toxic at high doses (Experiment 1), they appear non-toxic at expected environmental concentrations. These findings are summarized in Table 2 and Figure 4.

Table 1: Experiment 1

Test Article	Dose (mg/mL)	Mean Viability (%)	Standard Deviation (%)
DMTC	20	1.3	0.16
	100	1.6	0.17
	200	2.0	0.34
MBT-1	20	2.0	0.32
	100	3.3	0.26
	200	4.0	0.60
Negative Control	—	100.0	2.40 (DMTC) / 6.96 (MBT-1)
Positive Control	—	82.3	11.22

Table 2: Experiment 2

Test Article	Dose (mg/mL)	Mean Viability (%)	Standard Deviation (%)
DMTC	0.002	134.6	0.01
	0.01	107.2	8.15
	0.02	108.8	26.35
MBT-1	0.002	109.3	11.87
	0.01	108.7	11.05
	0.02	134.2	8.40
Negative Control	—	100.0	8.92 (DMTC) / 11.43 (MBT-1
Positive Control	—	63.5	2.55

Table 3. Globally Harmonized System Classification and Labeling of Chemicals, 10th Edition, 2023, UN, New York and Geneva.

Acute Toxicity	Category 1	Category 2	Category 3	Category 4	Category 5
Oral (mg/kg)	≤5	>5 ≤50	>50 ≤300	>300 ≤2000	Criteria • Anticipated oral LD_{50} between 2000 and 5000 mg/kg; • Indication of significant effect in humans;* • Any mortality at class 4;* • Significant clinical signs at class 4;* • Indications from other studies;* *If assignment to a more hazardous class in not warranted.
Dermal (mg/kg)	≤50	>50 ≤200	>200 ≤1000	>1000 ≤2000	
Gases (ppm)	≤100	>100 ≤500	>500 ≤2500	>2500 ≤5000	
Vapors (mg/l)	≤0.5	>0.5 ≤2.0	>2.0 ≤10	>10 ≤20	
Dusts & Mists (mg/l)	≤0.05	>0.05 ≤0.5	>0.5 ≤1.0	>1.0 ≤5	

DISCUSSION

One of the main problems with in vitro air testing in reconstructed tissues is extrapolating doses used in mg/mL (liquid) in the in vitro test to mg/m3 air concentrations used for animal and human exposure assessment.

Traditional approach. In Experiment 1, both chemicals were tested at high doses (20–200 mg/mL) following the standard MatTek protocol. These doses caused severe damage to the tissues, with very low cell viability (below 4% for all doses). This shows that both DMTC and MBT-1 are highly toxic at tested concentration levels and would be classified as hazardous under GHS Category 1 or 2 based on the MaTek prediction/conversion table.

One of the main problems of the in vitro air testing in reconstructed tissues is to extrapolate the doses used in mg/ml liquid in the in vitro test to mg/m3 air concentration used for animals and human sir pollutant assessment.

How to extrapolate these data to the real occupational exposure conditions?

Novel approach. 1) First, we extrapolated the observed Category 1 & 2 levels to a worst-case scenario LD50 concentration in air based on the following calculations. GHS hazard category 1 (worst-case scenario) defines category 1 for vapors at a maximum concentration of LC50 = 0.5 mg/L = 500 mg/m3 (Table 3).
2) The potential maximum concentrations in the occupational breathing zone were experimentally estimated in workplace settings due to a possible spill of the chemicals at 2.5 feet (worst-case scenario) and were measured at an average concentration of 0.5 mg/m3.
3) Based on that, the expected real concentration is at least 1,000-fold lower (500 mg/m3 ÷ 0.5 mg/m3 = 1,000-fold) than the LC50 for Category 1 chemicals (worst-case scenario).
4) Thus, the doses to be tested in vitro (in liquid vehicle) should be at least 1,000-fold lower than the highest dose tested in Experiment 1.
5) In Experiment 2, a second experiment was performed using significantly lower in vitro concentrations (0.002–0.02 mg/mL) at serial dilutions — representing a 1,000- to 10,000-fold reduction from the toxic in vitro doses used in Experiment 1 to better represent realistic workplace exposures.
At these levels used in Experiment 2, no toxic effects were observed. In fact, tissue viability stayed near or above normal (within the normal deviation of the measured MTT cell viability), and ED-25 values were above the highest tested dose (0.02 mg/mL), i.e., at least 1,000-fold lower than the toxic dose of hazardous class 1 (tested in Experiment 1). These results indicate that the chemicals are not harmful at the low concentrations expected in the human breathing zone after a potential spill.

CONCLUSION

These findings indicate that, despite the high toxicity of the tested chemicals, the levels of DMTC and MBT-1 expected in occupational settings (i.e., exposures likely to occur at work) do not pose a significant risk to human respiratory health. A novel approach was used in this study to correlate the in vitro concentration range (preprepared in a liquid vehicle) with real in vivo air concentrations in the occupational breathing zone. Additional studies with air pollutants from other GHS hazard categories are needed to confirm the validity of this approach.