

## CYP2E1 activity in Mexican workers occupationally exposed to low levels of toluene

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## Background

Volatile organic compounds (VOCs) are a class of chemicals widely used in several industrial applications, like painting, printing, footwear production, and synthetic or natural leather manufacture, among others.

People working at any of these workplaces may undergo a significant absorption of VOCs due to their high volatility and lipophilic properties.

One of the most used VOCs worldwide

T1V-TWA by ACGIH: 20 ppm

### Toluene

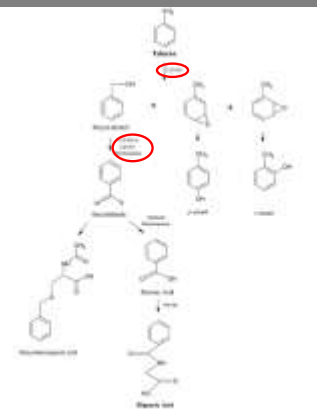
Acute exposure vs. Chronic exposure

High exposure levels

Low exposure levels

Significant although subtle damage in animals.

Production of ROS, associated with sympathetic and peripheral nerve dysfunction and sub-clinical hematological damage.



## CYP2E1

-CYP450 subfamily

-Highly conserved between species.

-Metabolizes many low- molecular weight potential toxic compounds including some VOCs .

- *In vivo* activity in humans: hydroxylation of chlorzoxazone (CHZ)  $\longrightarrow$  6 hydroxy chlorzoxazone (6OHCHZ).

- CHZ assay : Person fasting, ingests 500 mg of CHZ; 2 h later a blood sample is taken and plasma separated; measurement by HPLC of the parent drug and its metabolite. Activity (phenotype) is determined calculating the 6OHCHZ/CHZ ratio.

## Toluene occupational exposure and CYP2E1 activity in occupational settings

Lucas et al., 1999: Workers laboring at a footwear industry exposed to toluene and acetone showed lower CYP2E1 activity compared to controls.

Mendoza-Cantú et al., 2006: Toluene exposure levels were positive correlated with CYP2E1 mRNA in printing workers' lymphocytes.

## Study goal:

To explore the impact on CYP2E1 activity as a result of the occupational exposure to toluene and other VOCs in a group of tannery workers as compared to matched controls from the city of León Guanajuato, México.

## Material and methods

Participants:

- Male tannery workers specifically laboring at the "finishing" process, Exposed (E) group.
- Administrative workers from two universities, with no exposure to any VOC; Non exposed (NE) group.

After informed consent signature, data were obtained:

- Socio-demographic characteristics, general health status, tobacco and alcohol consumption.
- Height and weight.
- Individual environmental exposure levels for toluene, benzene and ethylbenzene (vapor diffusion monitors, GC/MS).
- Urinary hippuric acid levels by HPLC.
- After CHZ ingestion, 8 ml blood sample was collected and serum separated and CHZ and 6OHCHZ determined by HPLC.

## RESULTS

**Table 1**  
General characteristics of the study participants, exposed (E, n = 24) and non exposed (NE, n = 24).

Variable	Group	Mean	SD	Median	Maximum	Minimum
Age (years)	E	36.7	13.8	33.5	59	16
	NE	33.9	12.9	33	49	16
Body Mass Index (kg/m <sup>2</sup> )	E	25.3	3.2	24.7	30.3	17
	NE	26.09	4.03	25.01	38.8	15.2
Cumulative occupational exposure time (months)	E	67.8	88.1	45	25	300
	NE	NA	NA	NA	NA	NA

Figure 1. Individual air toluene exposure levels in the two groups. Levels were higher in E ( $p < 0.001$ , Mann Whitney  $U$  test).

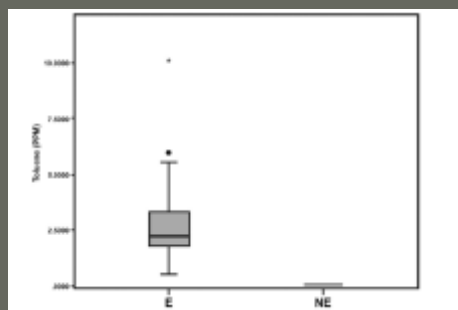


Figure 2. Simple linear regression between air toluene exposure level and post-shift urinary hippuric acid value in the E group. ( $r^2 = 0.13$ ,  $p = 0.087$ )

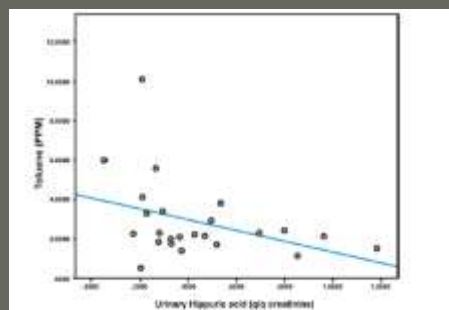


Figure 3 CYP2E1 enzymatic activity as determined by the 6-OH-CHZ/CHZ ratio in plasma after a 500 mg CHZ ingestion. Enzyme activity is lower in the E group ( $p < 0.05$ )

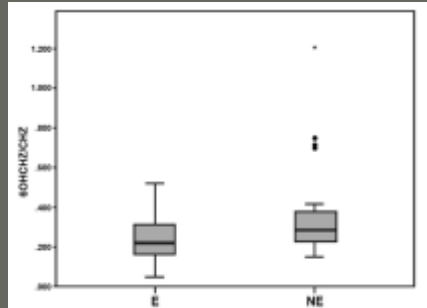
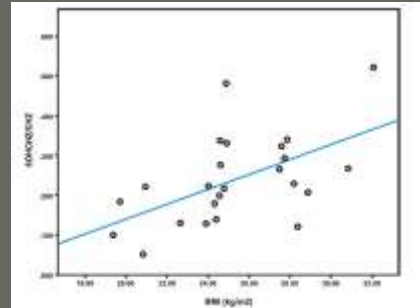


Figure 4. Simple lineal regression of CYP2E1 activity vs. BMI from persons of the E group. A positive correlation is observed ( $p < 0.005$ )



**Table 2**  
Regular alcohol consumption, cigarette smoking, and self-medication habits from the study participants.

Group	Alcohol consumption (%)	Smoking habit (%)	Practicing self-medication (%)
Exposed (n=24)	84 (n=20)	54 (n=13)	54 (n=13)
Non-exposed (n=24)	71 (n=17)	12.5 (n=3)	33 (n=8)

Figure 5. CYP2E1 activity in E smokers vs. NE nonsmokers. Enzyme activity is lower in the E subgroup ( $p < 0.005$ ).

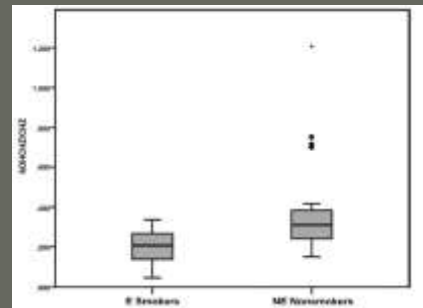


Fig. 6. (a and b) CYP2E1 activity comparison between subgroups (E vs. NE) according to smoking habit: (a) smokers ( $p = 1.0$ ); (b) non smokers ( $p = 0.19$ )

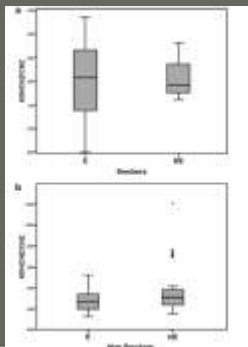
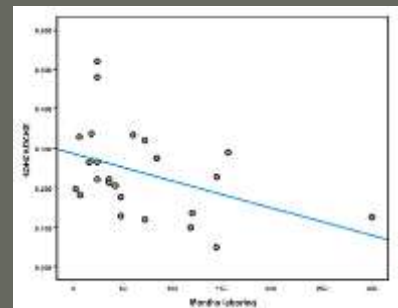


Fig. 7. Simple lineal regression of CYP2E1 activity vs. time laboring in E. As the time of laboring increases, enzymatic activity decreases ( $p < 0.05$ )



## Discussion

- Average toluene environmental levels were 25 times below the Mexican TLV-TWA, and 10 times below the recommended by ACGIH.
- Urinary hippuric acid did not increase as the toluene exposure levels did; coincident with several reports.
- CYP2E1 activity in E group behave similar like workers from a footwear factory exposed to toluene (Maximum level of exposure 341 ppm) and acetone (Lucas et al., 1999).
- Enzymatic activity and BMI: agrees with several reports; CYP2E1 association with cumulative exposure time: not reported before.

## Conclusion

- Occupational toluene exposure levels considered as "low " have a direct biological impact as judged by CYP2E1 activity.
- The impact of the cumulative time of exposure on CYP2E1 activity, and possibly also on regulation and expression, if confirmed, points to a chronic possibly irreversible effect of toluene on CYP2E1.

## Conclusion

- People occupationally exposed to any level of toluene should be monitorized for signs of subclinical damage.
- Those co-exposed to toluene and another CYP2E1 substrate deserve special attention.