Letter to the Editor

Correspondence re: K. Toide *et al.*, Aryl Hydrocarbon Hydroxylase represents CYP1B1, and not CYP1A1, in human freshly isolated white cells: trimodal distribution of Japanese population according to induction of CYP1B1 mRNA by environmental dioxins. *12:* 219–222, 2003

Letter

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A recent "Short Communication" by Toide *et al.* (1) suggested that CYP1B1 is induced by dioxins and that subjects exposed to dioxins from waste incinerators exhibit a trimodal inducibility distribution. In contrast, the expression of CYP1A1 could not be detected in the same subjects.

The authors analyzed the inducibility of CYP1B1 as ratio of CYP1B1 mRNA expression levels over the TEQ[2] concentration of dioxins in plasma. On the basis of the distribution of inducibility in the probit plot analysis, the authors identified three groups of subjects. Those with high and intermediate inducibility showed a significant correlation between CYP1B1 expression and dioxin TEQ concentration. We suggest an alternative interpretation: (*a*) the distribution of inducibility on the probit plot represents a log-normal and not a trimodal distribution. We propose that the amount of CYP1B1 expression as shown in the authors' Fig. 2*A*, as well as that of the CYP1B1/dioxin ratio in the probit plot of Fig. 2*B*, suggests a log-normal distribution. In addition, dioxin levels in the general population are known to be log-normally distributed (2). If log-normal data are not transformed, a probit plot can exhibit an apparent nonlinear pattern (3). (*b*) Besides the probit plot analysis, the correlations within the inducibility groups appear to be an artifact of the arithmetical procedure used by the authors. A within-group correlation between the same variables that are used to identify the groups (in this case, CYP1B1 expression and plasma dioxins concentration) will produce spurious positive results.

We report here results on CYP1B1 expression and dioxin TEQs in 71 subjects from Seveso, accidentally exposed to dioxins in 1976 (4). TEQ levels ranged between 7.4 and 181.0 pg/g lipid; CYP1B1 mRNA expression ranged between 137 and 148,939 copy number/10^7 copy number of actin mRNA. CYP1B1 expression was measured in freshly isolated lymphocytes with viability >75% by quantitative reverse transcription-PCR. Overall, there was no correlation between CYP1B1 expression and dioxin TEQ concentration ($n = 71$; $r = 0.08$; $P = 0.51$, Fig. 1*iii*). CYP1B1 mRNA, TEQ dioxin concentration, and CYP1B1/TEQ were log-normally distributed ($P = 0.47$, 0.82, and 0.53, respectively, Shapiro-Wilk test for normal data in log-transformed variables).

When we plotted the ratio CYP1B1/TEQ on the original scale, we obtained a curve similar to that observed by Toide *et al.* (Fig. 1*i*). However, when we transformed the distribution on a log scale, the probit plot was linear (Fig. 1*ii*). Thus, the curve in Fig. 1*i* could be spuriously perceived as trimodal or bimodal, whereas it was unimodal on a log scale. When we divided the subjects in three groups based on the plot on the original scale and then analyzed the correlation between CYP1B1 and TEQ within each group, we found a positive correlation in each group of subjects (Fig. 1*iii*). CYP1A1 expression was not assayed in freshly isolated lymphocytes because it is hardly detectable in uncultured cells (5). We measured both CYP1A1 and CYP1B1 expression in cultured lymphocytes and found no correlation with dioxin levels (4). We repeated the same analyses using multivariable regression models adjusted for possible confounders such as cell growth, experimental groups, and so forth and obtained similar results (4).

To illustrate how a spurious correlation between CYP1B1 expression and TEQ dioxin concentration can arise, we generated a series of pairs of independent log-normally distributed random variables and were able to reproduce the probit plots reported in Fig. 1*i* and *ii*. In Fig. 1*iv*, we report an example of the correlation results obtained by dividing the observations in three groups based on the simulated plot on the original scale. Although overall, there was no correlation ($n = 72$; $r = -0.05$; $P = 0.66$), in each subgroup, the correlation between simulated CYP1B1 expression and simulated TEQ was positive and statistically significant in the higher and intermediate groups as in Toide *et al.* (1) In conclusion, we failed to observe a correlation between dioxins concentration and CYP1B1 expression in the Seveso population. We suggest that the CYP1B1/TEQ ratio follows a log-normal distribution. Additional work is needed to evaluate the role of CYP1B1 in aryl hydrocarbon hydroxylase activity.

References

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Received 4/30/03; revised 6/20/03; accepted 6/23/03.

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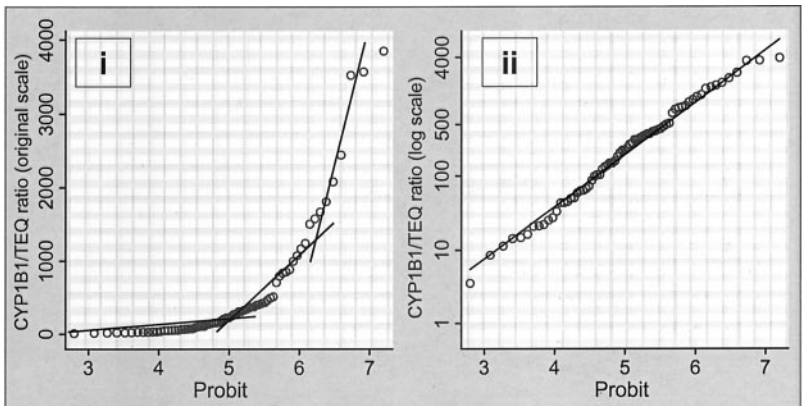
[2] The abbreviation used is: TEQ, toxic equivalent.



CYP1B1/TEQ ratio	iii SEVESO			iv RANDOM DATA		
	n	r	p-value	n	r	p-value
Any (all subjects)	71	0.08	0.51	72	-0.05	0.66
Low	36	0.49	0.003	40	0.25	0.12
Medium	26	0.68	0.0001	25	0.65	0.0005
High	9	0.78	0.01	7	0.79	0.04

Fig. 1. Probit-plot analysis of CYP1B1 mRNA expression (copies/10^7 copies of actin mRNA)/dioxin plasma levels (TEQ pg/g lipid) in a random sample of the Seveso population. Original (*i*) and log (*ii*) scales are shown. Correlation between CYP1B1 expression and dioxins concentration in the Seveso population (*iii*) and in a randomly generated data (*iv*) in subgroups of subjects based on the probit plot analysis on the original scale.

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Cancer Epidemiology, Biomarkers & Prevention

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Cancer Epidemiol Biomarkers Prev 2003;12:1116-1117.