

# Induction of *P*-450 in workers exposed to dioxin

William Halperin, Werner Kalow, Marie Haring Sweeney, Bing K Tang, Marilyn Fingerhut, Brent Timpkins, Kathleen Wille

## Abstract

**Objectives**—To examine the effects of occupational exposure to substances contaminated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on cytochrome *P*-4501A2 activity in a cross sectional medical survey.

**Methods**—The exposed workers had been employed at two chemical plants >15 years earlier in the manufacture of 2,4,5-trichlorophenol and its derivatives. The control group consisted of people with no occupational exposure to phenoxy herbicides and who lived within the communities of the exposed workers. A total of 58 workers and 125 unexposed controls participated in the analysis. Cytochrome *P*-450 activity was assessed with a test that measures caffeine metabolites in the urine. A ratio of metabolites of caffeine (CMR) constituted a measure of *P*-4501A2 activity.

**Results**—Compared with the control group in multivariate logistic regression, raised non-significant associations were found for three of four categories of TCDD in exposed workers (TCDD <20 pg/g, odds ratio (OR) 1.7, 95% confidence interval (95% CI) 0.6 to 5.0, TCDD 20–66, OR 0.3, 95% CI 0.0 to 1.7; TCDD 67–147, OR 2.3, 95% CI 0.6 to 8.8; TCDD ≥ 148, OR 3.1, 95% CI 0.8 to 12.5). We found a strongly significant association of CMR and urinary cotinine, a measure of smoking, and urinary free ethanol. We found weak non-significant associations between *P*-4501A2 activity and increased serum TCDD among workers.

**Conclusions**—The absence of an association between serum TCDD and cytochrome *P*-4501A2 may be due to the size of the study, insensitivity of the CMR to assess cytochrome *P*-4501A2 activity, or inadequate levels of exposure, although these were among the highest in human groups tested.

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Insights into the genetic basis for the toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)<sup>1</sup> began with the discovery<sup>2</sup> that TCDD was a potent inducer of cytochrome *P*-450<sup>3</sup> and that this induction was genetically controlled. This led to the recognition of a

cellular macromolecule, the Ah receptor, which specifically recognises and binds with TCDD. After processing and transport across the nuclear membrane TCDD binds to these specific sites on the DNA where it controls synthesis of cellular proteins that include cytochrome *P*-450 enzymes. In animals TCDD induces synthesis of two members of the *P*-4501A family of cytochromes *P*-450, *P*-4501A1 (aryl hydrocarbon hydroxylase (AHH)) and *P*-4501A2 (phenacetin-O-deethylase).<sup>4–8</sup> There is evidence for the existence of the Ah system in humans, which suggests that the mechanism involved in TCDD toxicity may be similar in humans and other susceptible species.<sup>9</sup> To describe the functional relation between exposure to TCDD and enzyme induction, and to determine the sensitivity of the system, we studied a group of controls and a group of workers with known serum concentrations of TCDD from former exposure to TCDD in the manufacture of herbicides and their precursors.

Grant *et al* described the conceptual basis for examination of profiles of the metabolites of caffeine as indicators of *P*-450 induction.<sup>10</sup> Caffeine (1,3,7 trimethylxanthine (137X)) is metabolised to 1,7 dimethylxanthine (17X) by *P*-4501A2.<sup>11–13</sup> The 17X is then oxidised to 1,7 dimethyluric acid (17U) by a *P*-450 that is not substantially inducible, or is further 7-demethylated by *P*-4501A2 to three other metabolites, 5-acetylamin-6-formylamino-3-methyluracil (AFMU), 1-methylxanthine (1X), and a derivative of 1X, 1-methyl-uric acid (IU). The ratio of (AFMU + 1X + IU)/17U in urine, caffeine metabolite ratio (CMR), has been proposed as an indicator of cytochrome *P*-450 activity inducible by polyaromatic hydrocarbons.<sup>14</sup> The CMR has previously been shown to be increased after environmental exposure to polybrominated biphenyls (PBBs)<sup>15</sup> and to cigarette smoke<sup>16</sup> but not by exposure to polyaromatic hydrocarbons in a foundry.<sup>17</sup> The CMR seems to be minimally related to sex and is significantly reduced by pregnancy and exogenous oestrogens; physical training increases the CMR as does ingestion of broccoli.<sup>11</sup>

## Methods

### STUDY POPULATION

The study population of exposed workers was recruited from current and former employees of two plants of the 12 included in the NIOSH mortality study of workers exposed to chemicals contaminated with TCDD.<sup>18</sup> At the

National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, Ohio, USA

W Halperin  
M H Sweeney  
M Fingerhut  
B Timpkins  
K Wille

Department of Pharmacology, University of Toronto  
W Kalow  
B K Tang

Correspondence to:  
Dr William Halperin,  
Division of Surveillance,  
Hazard Evaluations, and  
Field Studies, National  
Institute for Occupational  
Safety and Health, Centers  
for Disease Control and  
Prevention, 4676 Columbia  
Parkway, Cincinnati, OH  
45226, USA.

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two plants, TCDD exposure resulted from the manufacture of 2,4,5-trichlorophenol (TCP) and its derivatives. Exposure to TCDD occurred between 1951 and 1969 at the New Jersey plant, and for about two years between 1968 and 1972 at the Missouri plant. A cross sectional medical study of workers from the two plants was conducted in 1987 and 1988.<sup>19</sup>

A total of 586 workers, 490 from the New Jersey factory and 96 from the Missouri factory, were eligible for study. Of the 586 workers, 143 were dead (24%), and 43 (7.3%) were not traced, leaving 400 workers (68%) who were invited to participate in the study. A total of 357 of the eligible workers (89%) completed an occupational history; of those, 281 (70% of those eligible) also participated in the medical examination. A complete description of the study has been previously reported.<sup>19</sup> Twenty office workers with no appreciable occupational exposure were excluded from this analysis. Contractual arrangements for collection of urine samples for caffeine metabolism were concluded only after the study was well under way. Urine samples for caffeine metabolites and serum for measurement of TCDD were collected from 58 exposed workers. This represents 100% of the workers who were examined after the start of collection of urine for the analysis of caffeine metabolites.

Two hundred and sixty controls from the same neighbourhood, with no history of occupations in factories that manufactured chemicals contaminated with TCDD, participated in the medical examinations out of 325 controls who were interviewed.<sup>19</sup> Age (within five years), race, and sex matched controls were identified by a door to door screening of each worker's residential census record. Seventy five per cent of the controls represented the first, second, or third person identified as a potential candidate.

Of the 260 controls, serum TCDD was measured in only a sample of 79 people because only one of the sample controls had concentrations >20 pg/g and assays were expensive. Of the 260 controls, all 125 examined after contractual arrangements were completed were tested for CMR. Of the 125 controls tested for CMR, 24 had also been tested for TCDD. For epidemiological analysis, the 101 controls not tested for serum TCDD were assigned the median serum TCDD concentration found in the controls tested, 6.08 pg/g. This allowed the 101 controls not tested for TCDD to stay in the analysis and provide information on the association of CMR with other potential confounders, such as cotinine. Also, we estimated TCDD in samples where serum concentrations fell below the limit of detection. The estimated concentration was calculated by dividing the limit of detection of each sample by  $\sqrt{2}$ .<sup>20</sup>

#### LABORATORY METHODS

We have reported the methods of collection and preparation, analytical technique, and

quality control standards of the laboratory analyses for clinical chemistry and TCDD analyses.<sup>19,21</sup> For analysis of CMR, participants were asked to consume no less than one cup of coffee or tea or one can of soft drink and no more than six cups or cans on the day of urine collection. A nine hour urine collection began at 10 00 pm. At 7 00 am the collected urine was acidified to pH 3.5 and stored at -20°C. The set of frozen tubes was carried on dry ice by hand to the University of Toronto.

High performance liquid chromatography was used to measure urates and 1X.<sup>22</sup> The measurements of AFMU were performed according to Tang *et al.*<sup>23</sup> Cotinine was determined by the same procedure without modification of the TSK-20 column.

#### STATISTICAL ANALYSIS

For purposes of data exploration and ease of interpretation we first used simple stratified analyses. Given the known association of smoking cigarettes with CMR, smoking was chosen as one variable, worker *v* control was the other. Participants with urinary cotinine concentrations >0.0 were considered to be smokers. Variables considered included serum TCDD, age at the time of examination, alcohol-years (average number of alcoholic drinks consumed a day multiplied by the number of years alcohol was consumed), concentration of D-glucaric acid, pack-years (average number of cigarettes smoked a day divided by 20 multiplied by the number of years cigarettes were smoked), cotinine (g creatinine), weight (kg), race, self reported history of smoking (smoked within the past year), and self reported alcohol consumption (drank alcohol within the past year). Statistical comparisons were done with Student's *t* test for continuous variables and  $\chi^2$  test for categorical variables. All analyses were performed with SAS software.<sup>24</sup>

We used multivariate linear regression analyses to examine the association of CMR with serum TCDD and controlled for confounding variables. Models were constructed iteratively by the addition of predictor variables based upon biological reasoning. The first model explored the expected relation between CMR, the dependent variable, and serum TCDD; urinary cotinine was then added to the model. Subsequent models included variables that were predicted from the start to have some contribution to CMR. In these analyses, the distributions of CMR, serum TCDD, cotinine, etc were examined for normality. Log transformation of both the independent and dependent variables did not improve the normality of the residuals—that is, the difference between the observed and predicted values for each predictor—or the explanatory power of the regression equations ( $R^2$ ). Non-normalised variables are presented for ease of interpretation.

To model the relation of CMR with concentration of TCDD and to assess the contribution of other potential effect modifiers and confounders, such as cotinine, we conducted

Table 1 Characteristics of the study population by serum 2,3,7,8-TCDD category

	Controls	Workers:category of TCDD ((pg/g) (SD))				Total workers
		<20	20-66	67-147	148-1742	
No	125	19	13	13	13	58
Age (y; mean (SD))	56.4 (10.4)	54.1 (8.8)	53.9 (7.4)	56.9 (10.9)	56.0 (10.4)	55.1 (9.3)
Cups of coffee (mean (SD))	3.6 (2.0)	4 (2.4)	2.9 (3.0)	3.8 (1.6)	4.8 (4.1)	3.9 (2.8)
D-glucuronic acid ( $\mu$ mol/g creatinine; mean (SD))	23.7 (18.3)	26.2 (21.9)	22.8 (8.8)	20.4 (8.2)	16.0 (5.4)	21.9 (14.2)
CMR (mean (SD))	7.3 (4.4)	6.8 (4.0)	7.7 (4.8)	8.7 (7.5)	8.1 (3.2)	7.7 (5.0)
Pack-y (mean (SD))	28.9 (31.7)	34.2 (33.8)	35.2 (28.8)	33.1 (28.1)	38.4 (43.7)	35.1 (33.3)
Cotinine (mmol/g creatinine; mean (SD))	6.4 (12.9)	2.3 (6.8)	9.9 (14.0)	6.4 (12.9)	6.1 (12.0)	5.7 (11.4)
Weight (kg; mean (SD))	80.1 (14.5)	87.4 (20.0)	80 (14.0)	87.1 (15.1)	83.8 (11.0)	84.9 (15.8)*
White (%)	84.0	94.7	84.6	100	69.2	87.9
Smokers (current; %)	32.0	42.1	38.5	30.8	30.8	36
Alcohol-y (mean (SD))	61.9 (115.3)	60.9 (71.0)	23.9 (28.4)	63.0 (83.5)	41.4 (65.7)	48.5 (66.1)
Alcohol drinkers (current; %)	62.4	47.4	38.5	84.6	69.2	58.6
Ethanol ( $\mu$ mol/g creatinine; mean (SD))	150.9 (886.4)	38.1 (115.3)	3.8 (2.7)	4.5 (3.1)	89.1 (302.8)	34.3 (156.9)
TCDD $\ddagger$ (pg/g;mean (SD))	6.7 (4.3) $\ddagger$	11.4 (5.5)	35.0 (12.4)	100.9 (23.9)	548.2 (523.4)	157 (322)***

\*P &lt; 0.05; \*\*\*P &lt; 0.001 controls v workers;

 $\dagger$ P < 0.001 ANOVA analysis of TCDD categories, workers v controls; $\ddagger$ comparison of 58 exposed workers and 24 controls assayed for TCDD.

logistic regression analyses. For the logistic regression, CMR was dichotomised at the midpoint value (6.11) of the control population. Serum TCDD was included as a five level categorical variable and forced into all models regardless of significance. The controls consisted of 125 unexposed people, all of whom had TCDD concentrations <20 pg/g. The TCDD for workers was stratified into four levels (<20, 20-66, 67-147, and  $\geq$ 148 pg/g). The highest three categories of TCDD were determined by dividing the number of workers with concentrations of serum TCDD of  $\geq$ 20 pg/g into three groups nearly equal in size. After this, given the known association of cigarette smoking and enzyme induction, cotinine was entered as a categorical variable, but as the models did not converge, cotinine was entered into a substitute model as a continuous variable.

To further delineate the relation of TCDD

with CMR, other potential confounders and effect modifiers were examined by adding them one at a time to a base model containing TCDD, cotinine, and free ethanol. The additional variables were D-glucuronic acid (a measure of phenobarbital induced enzymes), age at examination, cups of coffee consumed, weight, years since last exposed, sex, race, and specific cohort (New Jersey or Missouri). Values for D-glucuronic acid and free ethanol were divided into quartiles and age and cups of coffee dichotomised based on the distribution in the control population. Tests for trends were conducted by entering TCDD as a continuous variable. An indicator variable was established to reflect whether TCDD concentrations in the controls were measured or estimated.

## Results

This study population consisted of 58 exposed workers and 125 controls from the same neighbourhood. The age of the study population ranged from 31 to 77 years. Table 1 shows the distribution of smoking, drinking, race, age, weight, and serum TCDD, by category of serum TCDD. The upper three groups had TCDD concentrations that were substantially higher than the controls and other unexposed populations that have been examined and live in industrialised countries.<sup>21</sup> As expected, mean TCDD was substantially higher in workers than in controls (157 v 6.7 pg/g; P < 0.001). Workers also weighed more than controls (84.9 v 80.1 kg; P < 0.05).

As smoking was a strong inducer of P-4501A2 and could mask an effect due to TCDD, we compared workers and controls by smoking (table 2). Smokers, regardless of exposure, drank more coffee. The difference was significant only in workers. The CMR was also significantly higher in smokers among workers (10.9 v 6.8; P < 0.05) and controls (11.6 v 5.9; P < 0.001). The association of smoking and CMR served to validate the performance of the CMR assay in this study. When smokers among the workers and

Table 2 Comparison of workers and controls by smoking

Variable (SD)	Workers	Controls
Smokers: $\ddagger$		
No	13	31
TCDD (pg/g)*	217.2 (471.5)	5.3 (2.1)
Age at examination	51.5 (8.8)	54.9 (9.9)
Alcohol (y)	41.0 (47.7)	81.5 (141.0)
Cups of coffee/day	5.7 (2.06) $\ddagger\ddagger$	4.2 (2.5)
DGA ( $\mu$ mol/g creatinine)	24.1 (7.3)	24.3 (22.3)
CMR	10.9 (3.4) $\ddagger\ddagger$	11.6 (5.5) $\ddagger\ddagger\ddagger$
Pack-y	52.7 (20.8) $\ddagger\ddagger$	43.9 (30.2) $\ddagger\ddagger$
Weight (kg)	83.3 (11.0)*	74.6 (13.2) $\ddagger\ddagger$
Cotinine (mmol/g creatinine)	25.6 (7.8) $\ddagger\ddagger\ddagger$	26.0 (12.8) $\ddagger\ddagger\ddagger$
White (%)	100	87
Smoker (current) (%)	92.3	93.5
Drinker (current) (%)	69.2	54.8
Non-smokers: $\ddagger$		
No	45	94
TCDD (pg/g)	139.6 (269.5)**	7.1 (4.7)
Age at examination	56.1 (9.2)	56.9 (10.6)
Alcohol-y	50.7 (71)	55.4 (105.6)
Cups of coffee/day	3.4 (2.8)	3.3 (1.8)
DGA $\mu$ mol/g creatinine	21.2 (15.7)	23.5 (16.9)
CMR	6.8 (5.0)	5.9 (2.8)
Pack-y	30.0 (34.6)	24.0 (30.8)
Weight (kg)	85.3 (17.0)	81.9 (14.5)
Cotinine (mmol/g creatinine)	ND	ND
White %	84.4	83.0
Smoker (current %)	20.0	11.7
Alcohol-y	0.55 (0.50)	0.65 (0.48)

\*P < 0.05; \*\*P < 0.01 workers v controls;  $\ddagger$ P < 0.05;  $\ddagger\ddagger$ P < 0.01;  $\ddagger\ddagger\ddagger$ P < 0.001 smokers v non-smokers; ND = not detectable;  $\ddagger$ Smokers defined by urinary cotinine > minimum detectable.



Caffeine metabolite ratio (CMR) *v* concentrations of the dioxin TCDD in exposed workers (●) and controls (○).

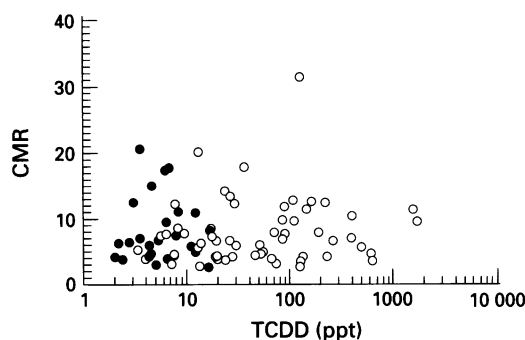


Table 3 Mean (SD) concentrations for controls with measured and estimated TCDD

Variable	Measured TCDD	Estimated TCDD
No	24	101
Serum TCDD (pg/g)	6.7 (4.3)	—
CMR	8.2 (5.1)	7.1 (4.2)
Age at examination	51.8 (11.0)	57.5 (10.0)
Alcohol-y	63.5 (87.0)	61.5 (121.5)
Cotinine (mmol/g creatinine)	6.1 (12.9)	6.5 (13)
Alcohol (current; %)	86	56
Free ethanol (μmol/g creatinine)	546.7 (1914.1)***	56.9 (291.5)
D-glucaric acid (μmol/g creatinine)	19.1 (6.6)***	24.8 (20.0)
Cups of coffee/day	3.9 (1.5)	3.5 (2.1)
White (%)	83	84
Weight (kg)	79.8 (17.0)	80.1 (13.9)
Smoking (current; %)	33.3	32
Pack-y (cigarette)	26.4 (30.8)	29.6 (32.0)

\*\*\*P < 0.001.

Table 4 Predictors of CMR in models of linear regression analyses

Variable	Model 1 β (SE) P	Model 2 β (SE) P	Model 3 β (SE) P
Intercept	7.39 (0.3) 0.0001	6.21 (0.33) 0.0001	12.7 (2.46) 0.0001
TCDD (pg/g)	0.0012 (0.002) 0.5	0.0006 (0.0015) 0.68	0.0009 (0.001) 0.05
Cotinine (mmol/g creatinine)	—	0.194 (0.023) 0.0001	0.2 (0.025) 0.0001
Free ethanol (μmol/g creatinine)	—	—	0.0012 (0.000) 0.003
D-glucaric acid (μmol/g creatinine)	—	—	-0.017 (0.0168) 0.32
Age	—	—	-0.039 (0.029) 0.18
Weight	—	—	-0.043 (0.019) 0.03
Cups coffee/day	—	—	-0.14 (0.13) 0.27
R <sup>2</sup>	0.0025	0.279	0.350

Table 5 Predictors of CMR\* in logistic regression analysis model

Variable	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Controls:			
TCDD (<20 pg/g)	1	1	1
Workers:			
TCDD (<20 pg/g)	1.0 (0.4 to 2.5)	1.4 (0.5 to 3.9)	1.5 (0.5 to 4.4)
TCDD (20–66 pg/g)	0.6 (0.2 to 2.0)	0.2 (0.0 to 1.3)	0.3 (0.0 to 1.7)
TCDD (67–147 pg/g)	1.6 (0.5 to 5.1)	1.8 (0.5 to 6.4)	2.3 (0.6 to 8.8)
TCDD (>148–1742 pg/g)	2.2 (0.6 to 7.6)	2.7 (0.7 to 9.9)	3.1 (0.8 to 12.5)
Cotinine (mmol/g creatinine)	—	1.2 (1.1 to 1.3)	1.2 (1.1 to 1.3)
Free ethanol (<2.64 μmol/g creatinine)	—	—	1
Free ethanol (2.64 < 4.19 μmol/g creatinine)	—	—	0.9 (0.3 to 2.3)
Free ethanol (>4.19 < 7.4 μmol/g creatinine)	—	—	1.3 (0.5 to 3.4)
Free ethanol (>7.4 μmol/g creatinine)	—	—	4.9 (1.7 to 13.9)

\*Case defined as CMR > 6.11.

controls were compared, there were significant differences in weight.

The 24 controls who were tested for TCDD compared with the 101 who were estimated for TCDD (table 3) had significantly higher concentrations of free ethanol and lower concentrations of D-glucaric acid ( $P < 0.001$ ). As the controls for this test were chosen consecutively, the difference may represent either a chance occurrence or a temporal pattern in the characteristics of controls associated with their date of examination.

No association of CMR with TCDD was evident from a scattergram (figure) of only these variables. In multivariate linear regression analyses (table 4), there was almost no relation of CMR with serum TCDD ( $\beta$  coefficient 0.00012,  $P = 0.5$ ). We found a significant positive association between urinary cotinine, an indication of current cigarette smoking and CMR ( $\beta$  coefficient 0.194,  $P = 0.0001$ ). Free ethanol, an indication of recent alcohol consumption, was positively associated with CMR ( $\beta$  coefficient 0.0012,  $P = 0.003$ ). Weight and CMR were negatively associated ( $\beta$  coefficient -0.043,  $P = 0.03$ ). A model that contained cotinine and serum TCDD predicted 28% of the variation in CMR, compared with only 0.25% for TCDD alone. The intercept (6.2 in model 2), a measure of the non-induced or constitutive enzyme activity in non-smokers was consistent with studies in other populations and in other laboratories.<sup>17 25</sup>

Table 5 shows the results of analysis by logistic regression. In model 1, four groups of exposed workers were compared with the controls. Although odds ratios ORs were high for three of the four exposed groups, confidence intervals (CIs) of the ORs were broad and none exceeded expectancy, an OR of 1. With the known relation of CMR to smoking, in model 2 cotinine was added as a predictive variable. The OR (1.2; 95% CI 1.1 to 1.3) indicates a 20% increase in CMR for each unit rise in cotinine. The addition of the cotinine to the model did not substantially change the ORs for concentrations of TCDD, which suggests little confounding between these two variables. Urinary free ethanol was entered into model 3 because of variations in the concentration in free ethanol found in the stratified analyses. The OR increased with concentration of free ethanol, with the highest quartile significantly associated with CMR (OR 4.9; 95% CI 1.7 to 13.9). Other variables were then added one at a time (models 4–11; data not shown) to a model that included all values of serum TCDD, cotinine, and the highest concentration of free ethanol. These included D-glucaric acid, age at time of the examination, cups of coffee consumed before the examination, weight, years since last exposure to TCDD, sex, race, and whether a member of the New Jersey or Missouri cohort. None of these additional variables were significant predictors of CMR. In no model tested was the indicator variable for measured *v* estimated concentration of TCDD a significant predictor of CMR. To

assess a trend in association between serum TCDD and CMR, TCDD was entered as a continuous variable in a model that included cotinine as a continuous variable, and values of urinary free ethanol. No trend was found; the OR for TCDD was 1.0010 (95% CI: 0.999 to 1.003).

### Discussion

This study showed essentially no relation between serum TCDD and induction of *P*-450 enzyme as measured by the CMR. In comparison, and consistent with other reports,<sup>25</sup> a strong association was evident between induction of *P*-4501A2, as measured by CMR, and cigarette smoking as measured by urinary cotinine. The only indication of an association between CMR and TCDD was faint and was found in logistic regression. In logistic regression, for three of four categories of exposed workers defined by increasing concentrations of serum TCDD, the OR was high. The increases were not significant.

There are several explanations for these findings. Size was a main limitation of this study. When the study population is divided for analysis into groups by exposure, there are small numbers of participants in the highly exposed groups. Although these chemical workers currently have among the highest serum concentrations of TCDD that have been found in human populations, and they have carried these burdens for >15 years since last exposure, their numbers may be too small in this study to accurately reflect the association between TCDD and CMR. The limited power to detect an association between TCDD and CMR is particularly worrying because of the increased ORs in the workers of the two highest categories of exposure to TCDD (table 5).

This population showed a strong relation of CMR to cigarette smoking, to which TCDD, as an inducer, can be compared. The strength of association between CMR and cigarette smoking ( $R^2$  0.269) is lower than previously reported ( $R^2$  0.81),<sup>16,25</sup> which may reflect the older age of our study population.

Other biases that may have obscured a strong relation between CMR and serum TCDD include measures of exposure and measures of response. The measure of exposure among the workers was their serum TCDD. In our population there was a strong correlation ( $R$  0.7,  $P < 0.001$ ) between serum TCDD and duration of employment in an exposed job<sup>18</sup>; hence substantial error in measurement of serum TCDD is unlikely. The measure of response in this population was the CMR, which has been used in several earlier studies that investigated the relation of enzyme induction and cigarette smoking,<sup>15,27</sup> and exposure to polycyclic aromatic hydrocarbons in foundries,<sup>17</sup> diet, exercise, and oestrogens.<sup>11</sup> These studies showed strong and consistent relations between smoking cigarettes and CMR, as was evident in our analysis. It is possible that the CMR, although an extremely practical test and clearly sufficiently sensitive

to detect the strong relation of cigarette smoking with enzyme induction, may not be sufficiently sensitive and specific in our study population to measure *P*-4501A2 induction associated with TCDD. There has been substantial discussion about the enzymes actually reflected by the caffeine urine test<sup>27</sup> compared with the caffeine breath test.<sup>15</sup> There has been further discussion about the most appropriate caffeine metabolites to measure in the urine test.<sup>26</sup> As was done in a comparison of two methods of assessing the effect of PBBs on *P*-450 induction,<sup>15</sup> future studies might include comparisons of the sensitivity of alternative methods to assess cytochrome *P*-4501A2 induction.<sup>26,27</sup>

The stronger relation between cigarette smoking than TCDD on CMR suggests two conclusions. Cigarette smoking may be a stronger inducer of *P*-4501A2 than is TCDD in the highly exposed participants in this study, or CMR may measure another enzyme that is stimulated by cigarette smoking and that is distinct from *P*-4501A2. This possibility is very unlikely, based on *in vitro* studies with human *P*-450s derived from DNA which seemed to account for most or all of the caffeine metabolising capacity of microsomes of the human liver.<sup>28</sup>

Another possible explanation for the weak association between serum TCDD and CMR is that the dose of TCDD in this population, although among the highest found in humans,<sup>21</sup> may not be sufficient in comparison with experimental doses given to animals to elicit an induction in *P*-450. It has been suggested that humans are among the least sensitive species for induction of hepatic enzyme.<sup>29,30</sup>

A final alternative is even more speculative. This study differs from experiments on laboratory animals in its time frame. The workers in this population had their last occupational exposure to TCDD 15 to 37 years before this study was performed and because of the long half life of TCDD, can be considered as having been continuously exposed during employment and subsequently. In comparison experimental studies usually measure enzyme induction comparatively soon after TCDD exposure. Might it be possible that TCDD induction of *P*-4501A2, in parallel to well understood hormones, is self regulating? Perhaps the induction of *P*-4501A2 leads to increasing unresponsiveness *in vivo* to the continued potency of TCDD as an inducer. Some evidence against this hypothesis is the lack of negative association between CMR and years since last exposure.

In summary, this study showed the inability of serum TCDD in comparison with cigarette smoking, to raise CMR. Numerous methodological and mechanistic explanations are possible. These include questions about the sensitivity of the CMR, the responsiveness in humans of *P*-4501A2 induction by TCDD, the limited size of this study, or the inadequate exposure in this population to elicit a response, even in a group of the most highly occupationally exposed people.

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