THE IMPACT OF DIOXINS ON ANTIPYRINE METABOLISM IN RUSSIAN FIREFIGHTERS EXPOSED TO COMBUSTION PRODUCTS DURING A CABLE FACTORY FIRE

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Introduction

The current study examines antipyrine metabolism and dioxin levels among firefighters who were involved in a catastrophic warehouse fire that took place in 1992 near the town of Shelekhov, Russia. During the event, firefighters avoided using respiratory protection because of concerns about the safety of individual oxygen kits in the presence of flammable technical oil[1]. As a result, they may have been exposed to dioxins when they inhaled smoke during the combustion of more than 1000 tons of polyvinylchloride (PVC), polyethylene and other plastics.

Materials and methods

A total of forty men were examined with thirty coming from the cohort of 165 firefighters formed in 2003 to study the impact of the Shelekhov fire. They include ten selected from Group 2 which consists of firefighters who developed the Shelekhov Syndrome Complex soon after their participation at the fire (Shelekhov firefighters-SSC); ten from Group 3 which consists of firefighters who participated in the Shelekhov fire but did not develop the syndromes (Shelekhov firefighters); and ten were from Group 4, firefighters who did not participate in the 1992 Shelekhov fire (Firefighter controls). An additional ten men formed a control group of individuals who have never worked as firefighters (Group 5; Non-firefighter controls). The formation of groups was complicated due to the limited number and accessibility of candidates (due to change of residence, death and other reasons) as well as reluctance to participate in the examination (over 20 declined). Men were screened to ensure their body mass index (BMI) and ages were within comparable ranges. Informed consent (approved by the Biomedical Ethics Committee of East-Siberian Scientific Center of Siberian Branch of Russian Academy of Medical Sciences, Irkutsk) was obtained from all participants.

After overnight fasts, each participant provided 40-50 ml of blood from which serum was obtained using a standard procedure. Seven polychlorinated dibenzo-p-dioxin (PCDD), 10 polychlorinated dibenzofuran (PCDF), and 12 polychlorinated biphenyl (PCB) congeners were analyzed in each of the samples at the A.N. Severtsov Institute of Ecology and Evolution (Moscow). The methods for sample preparation, clean up and analysis are described in Laboratory SOP "Guideline on identification and isomer specific determination of polychlorinated dibenzo-p-dioxins, dibenzofurans, biphenyls and organochlorine pesticides in biological samples by HRGC-HRMS", which followed US EPA 1613, 1668, and U.S. Centers for Disease Control and Prevention (CDC). The analyses of PCDD/Fs fractions were performed by gas chromatography/high-resolution mass spectrometry using Thermo Finnigan MAT 95XP at resolution 10000, equipped with a SGE BPX-5 column (30 m x 0.22 mm x 0.25 μm) for planar fraction and SGE HT-8 column (30 m x 0.25 mm x 0.25 μm) for non-planar compounds. Each analytical run consisted of a method blank and four unknown samples. All solvents, sorbents and reusable glassware were tested to ensure the absence of contaminants and interference.

The system of toxicity equivalent factors developed by the World Health Organization (WHO) in 1998 (for humans) and 2005 were used to calculate total toxicity equivalent (TEQ)[2,3]. Measurements below the limit of detection (LOD) were assigned a value representing the level of detection divided by the square root of 2 as recommended by the CDC. We estimated body burden with TEQs and the following formula: %Lipids = 495/(1,0324 – 0,19077(log(waist – neck)) + 0,15456(log(height))) – 450)[4].

The current study used pharmaceutical purity antipyrine (AP; Fluka) as a metabolic probe to measure the activity of hepatic oxidative enzymes. Emphasis is placed on 3-hydroxymethylantipyrine (3HMAP), the metabolite with the greatest dependence on cytochrome P4501A2 (CYP1A2) activity. The protocol and justification for using AP as a substrate for assessment of CYP1A2 activity has been previously described[5]. Briefly, because AP has

not been used as an analgesic in recent years, there is no possibility that participants are exposed outside of the study. AP metabolism was assessed by HPLC performed on urine samples obtained from 38 participants using phenacetin (Aldrich) as an internal standard. Two individuals were excluded because of medical conditions.
Smoking status was determined by the excretion of urinary cotinine measured with a Cotinine Direct ELISA Kit (Bio-Quant, Inc.) using a universal microplate reader ELx800 (Bio-Tek Instruments, Inc.). Urine samples were obtained prior to the blood collection and after ingestion of AP.
Statistical analysis was performed with STATISTICA 6.1 (StatSoft).

Results and discussion:
Selected demographic characteristics consisting of disability status, smoking status, and dioxin levels are presented in Table 1 by group. The groups have comparable Age and BMIs while their smoking and disability status differ. All firefighters in Group 2 are disabled due to their participation in the Shelekhov fire whereas 4 firefighters in Group 3 are disabled (only one of the disabilities is due to participation in the Shelekhov fire). The average TEQ values in the groups are not statistically different. There is a trend for increasing TEQ values for both indices in the following order: Non-firefighter controls (Group 5) – Shelekhov firefighters-SSC (Group 2) – Shelekhov firefighters (Group 3) – Firefighter controls (Group 4), which corresponds to their likely recent exposure to smoke. Currently, the dioxin levels in the blood of firefighters highly exposed to combustion products in 1992 are comparable to the values measured in working firefighters who did not participate in the fire suppression at the cable factory. Shelekhov firefighters (Group 3) have significantly higher body burdens of dioxins than the Non-firefighter controls (Group 5). In a separate report, we found that working firefighters have higher levels of 1,2,3,4,6,7,8-HpCDD, a congener associated with combustion processes[6].

Table 1. Demographic characteristic and dioxin level of the study participants (Mean, min–max)

Parameters	Group 2 n=10	Group 3 n=10	Group 4 n=10	Group 5 n=10	p^A
Age, yrs	45 (39-54)	45,1 (33-52)	42 (35-51)	45,4 (38-52)	0,776 / 0,483
BMI, kg/m^2	27,3 (21,8-34,3)	27,3 (21,7-36,0)	26,6 (23,3-29,6)	25,4 (21,1-30,3)	0,850 / 0,585
Disabled, #	10	4 (3)[B]	0	0	
Employed as firefighters when examined, #	0	4	9	0	
Operational experience as firefighters, yrs	13 (9-17)	16,5 (9-25)	16,8 (9-29)	0	
Current smokers, #	6	3	7	4	
Cotinine, ng/ml	9145 (5-38213)	7098 (6-51154)	8499 (4-25311)	4369 (1-18792)	0,304 / 0,262
Total WHO-TEQ^{98}, pg/g lipids	34,3 (21,2-52,7)	37 (23,3-62,3)	41,7 (15,8-80,6)	28,2 (14,3-45,6)	0,387 / 0,850
Total WHO-TEQ^{05}, pg/g lipids	20,1 (12-30,3)	22,3 (14,5-34,3)	25,3 (9,2-46)	17,3 (6,1-34,5)	0,334 / 0,494
Body burden^{98} , ng	666 (287-1332)	708 (388-1737)	795 (300-2129)	465 (155-811)[3]*	0,248 / 0,133
Body burden^{05}, ng	396 (165-877)	***415*** (241-851)	476 (173-1148)	***285*** (100-695)[3]**	0,153 / ***0,016***

Note. [A] – Significance level (p) in intergroup comparison: one-way analysis of variance (Kruskal-Wallis ANOVA/ Median Test); [B] – in parenthesis, the number of the firefighters whose disability is not related to the fire suppression at the cable factory in 1992; [3]*, [3]** – compared to Group 3, p=0,069 and p=0,016 respectively: Mann-Whitney U test.

Urinary excretion of 3HMAP, the metabolite most closely related to CYP1A2 activity differed between groups (p<0,1) as shown in Table 2. Current firefighters (Group 4) have significantly higher levels of 3HMAP excretion (p<0,05) than either Shelekhov firefighters (Group 2 and Group 3) or Non-firefighter controls (Group 5). Shelekhov firefighters with SSC (Group 2) excreted the lowest levels of 3HMAP, a finding that is consistent with earlier analyses[5]. Smoking may play a role since the groups with the highest excretion of 3HMAP (Group 4 followed by Group 2) also had the highest number of smokers.

Table 2. Antipyrine metabolism by Group

Parameters[A]	Group 2 n=10	Group 3 n=10	Group 4 n= 8	Group 5 n=10	p[B]
SUM	68,4 (49,6-73,9)	54,5 (44,0-66,0)	73,5 (62,4-83,3)	60,7 (36,2-78,2)	0,621 / 0,637
NAP	15,3 (11,1-19,1)	11,4 (10,3-15,3)	14,2 (12,4-16,2)	13,0 (8,7-18,6)	0,450 / 0,825
4HAP	27,8 (24,67-34,7)	23,3 (20,0-27,8)	29,3 (23,8-33,5)	26,4 (14,0-39,8)	0,806 / 0,475
3HMAP	***17,9*** (11,9-28,3)	***22,7*** (14,4-27,0)	***28,2*** [2, 3] (22,2-33,4)	***18,34*** [4] (14,7-26,5)	0,091 / 0,424
AP	2,9 (1,3-4,3)	3,0 (2,1-3,4)	2,6 (2,0-3,0)	4,3 (2,1-4,9)	0,598 / 0,825

The data are expressed as the median value (Me) and interquartile range (upper limit of the lower quartile, LQ; and lower limit of the upper quartile, UQ); [A] – Sum of metabolites, NAP – norantipyrine, 4HAP – 4-hydroxyantipyrine, 3HMAP and AP as % of total AP dose; [B] – Significance level (p) in intergroup comparison: one-way analysis of variance (Kruskal-Wallis ANOVA/ Median Test); [2, 3, 4] – compared to Group 2, Group 3 and Group 4, respectively: p<0,05, Mann-Whitney U test.

Table 3 shows the correlation coefficients between the 3HMAP and exposure by group. Urinary cotinine levels are highly correlated to 3HMAP. The groups with the highest correlation between 3HMAP and body burdens were the firefighter controls (Group 4) and the Shelekhov firefighters (Group 3), the majority of whom are currently working as firefighters.

Table 3. Spearman bivariate correlations between 3HMAP, urinary cotinine, TEQ^{98}, and body $burden^{98}$

Pair, n	All n=38	Group 2 n=10	Group 3 n=10	Group 4 n=8	Group 5 n=10
3HMAP-Cotinine	0,695**	0,733*	0,624*	0,810*	0,673*
3HMAP-Body burden	0,127	0,139	0,467	0,404	-0,200
3HMAP-TEQ^{98}	0,071	0,067	0,079	0,238	-0,067
Cotinine-Body burden	0,258	0,055	0,406	0,238	0,224
Cotinine-TEQ^{98}	0,167	0,018	0,055	0,143	0,297

* – p<0,05; ** – p<0,001.
3HMAP as % of total AP dose; urinary cotinine – ng/ml; TEQ^{98} – pg/g lipids; body $burden^{98}$ – ng.

Regression analysis was performed for 38 individuals to evaluate the validity of the metabolic test indices as potential biomarkers of effect. Antipyrine test indices were the dependent variables; age, cotinine level and total body $burden^{05}$ were chosen as predictor variables (Table 4).
The analysis indicates that the models obtained for SUM, 4HAP and 3HMAP adequately describe the linear relationship between the variables. The best fit was obtained for 3HMAP based on the coefficient of multiple determination (R^2=0,707). The associations between dioxin body burden and antipyrine test indices are not significant although the β_3 for 3HMAP has a positive value whereas the corresponding regression coefficients for SUM, NAP and 4HAP are negative.
The models indicate with different degrees of significance the inverse association between main AP metabolites

level and the age of the individuals. This is consistent with the recognized age-related decrease of functional activity of most CYPs, especially CYP1A2[7]. Assessment of cotinine permitted the evaluation of the contribution of tobacco smoking which was maximal for the 3HMAP model (0,687, p=0,000). The significant contribution of cotinine in defining the formation of 3HMAP and 4HAP is consistent with data on CYP1A2 induction during tobacco smoking[8].

Table 4. Multiple linear regression analysis

Parameters	Age, yrs		Cotinine [A]		Total TEQ[05], pg/g lipids		Model adjusted		
	Standardized								
	β_1	p_1	β_2	p_2	β_3	p_3	R^2	F	p
SUM	-0,222	0,120	***0,566***	0,000	-0,006	0,965	***0,606***	6,576	<0,0012
NAP	***-0,339***	0,046	0,128	0,434	-0,012	0,944	0,363	1,716	<0,1821
4HAP	-0,175	0,250	***0,489***	0,002	-0,049	0,748	***0,517***	4,140	<0,0132
3HMAP	-0,114	0,367	***0,687***	0,000	0,048	0,705	***0,707***	10,964	<0,0001
AP [A]	-0,133	0,419	-0,265	0,107	0,285	0,092	0,367	1,769	<0,1716

[A] – decimal-log-transformation;
R^2 – coefficient of multiple determination, F- and p-values for regression equation;
p_n – p-values for standardized regression coefficients β_n.

These results suggest that exposure to dioxins is a consequence of occupational exposure as a firefighter. The model for the formation of the 3HMAP metabolite makes it possible to evaluate the contribution of dioxin while distinguishing the impact of age and tobacco smoking upon the functional activity of cytochrome P4501A2. Further analysis will more fully incorporate the impact of long lived congeners, the duration of exposure, and the contribution of polymorphisms of *CYPs* gene catalyzing AP metabolism reactions.

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