Lipid metabolism and neuropsychological follow-up study of workers exposed to 2,3,7,8-tetrachlordibenzo-p-dioxin

Abstract Objective: More than 35 years ago, during 1965–1968, in the former Czechoslovakia, approximately 80 persons became ill due to occupational exposure to 2,3,7,8-tetrachlordibenzo-p-dioxin (2,3,7,8-TCDD). The objective of this study was to investigate the incidence of disorders related to occupational exposure to 2,3,7,8-TCDD. Methods: Most subjects in the group of 12 former 2,3,7,8-TCDD workers (mean age 56.8 years, exposure 10 days to 23 months) still suffer from disturbances of lipid metabolism, psychic disorders, chloracne, and/or nervous system lesions. All workers were given internal, neuropsychological and ophthalmological examinations. Blood cholesterol and triglycerides were measured, and the common carotid artery was examined by ultrasound (B-mode), with the intima-media thickness (IMT) also being measured. Findings were compared with the 2,3,7,8-TCDD level in 1996. Results: Nine of the 12 previously exposed workers had elevated plasma lipids, and hyperlipidaemia was statistically more frequent in patients with higher 2,3,7,8-TCDD levels (P=0.03). Subject 1, with the highest 2,3,7,8-TCDD plasma level, had 80% stenosis of the diameter of the carotid artery, which needed acute surgery. Besides him, seven persons had atherosclerotic plaques in the carotid arteries. The mean IMT in the group was 0.85 mm (SD±0.19); the normal value is 0.62 mm. Eight subjects had degenerative changes of the ocular fundus. Chloracne was still present in two persons. Neuropsychological findings were assessed as normal only in three persons with lower 2,3,7,8-TCDD plasma levels in 1996. Mean 2,3,7,8-TCDD plasma level in 1996 was 256 pg g–1 fat (range 14–760). Conclusion: Hyperlipidaemia, atherosclerotic plaques, increased IMT, ischaemic heart disease and neuropsychological disturbances were frequent in this group of former 2,3,7,8-TCDD workers. Hyperlipidaemia might have played an important role in most of these disorders. The level of 2,3,7,8-TCDD correlated with the highest level of triglycerides (P=0.02) and cholesterol (P=0.01) that was found during the 35-year follow-up. This group belongs to the most heavily 2,3,7,8-TCDD-exposed groups of workers, because the mean estimated concentration at the time of intoxication was approximately 5,000 pg g–1 plasma fat.

Keywords 2,3,7,8-TCDD · Hyperlipidaemia · Atherosclerosis · Neuropsychological changes · Chloracne

Objective

The history of intoxication of approximately 80 Czech workers due to occupational exposure to 2,3,7,8-tetrachlordibenzo-p-dioxin (2,3,7,8-TCDD) in herbicide production (butylester of trichlorphenoxyacetic acid) in a chemical factory in Spolana in Central Bohemia is more than 35 years old. There was no accident, but
because of a higher temperature and pressure during production in 1965–1968, an uncontrolled decomposition reaction occurred and 2,3,7,8-TCDD originated as an intermediate product (Pazderová-Vejlupková et al. 1981). Several persons were also exposed during the subsequent clean up of the plant. 2,3,7,8-TCDD was later detected in the wall paint of the factory and in the soil. It was also found in the final product. The concentration in the blood of exposed workers was first measured in 1996, i.e. approximately 30 years after the exposure, and the mean level of 256 (SD ± 139) pg g⁻¹ fat confirmed a very high level of exposure. Back-calculation of the levels to the time of the exposure, using a 2,3,7,8–TCDD half-life of 7.1 years brings an estimated average 2,3,7,8-TCDD concentration in 1967–68 of approximately 5,000 pg g⁻¹ plasma fat; the maximum concentration in subject 1 in 1968 could have reached approximately 14,000 pg g⁻¹ plasma lipid (Pelcová et al. 2001).

In 1996, there was a significant positive correlation of 2,3,7,8-TCDD with blood cholesterol, triglycerides, and beta-lipoproteins. In 2001, an examination focussing on atherosclerotic damage was performed on a group of 12 former 2,3,7,8-TCDD workers, i.e. about 35 years after the exposure.

In parallel, a neuropsychological follow-up examination was executed, because significant correlation was also found in 1996 between neuropsychological variables and the plasma level of 2,3,7,8-TCDD. Other significant correlations were seen between neuropsychological variables and the levels of triglycerides and cholesterol in most measurements throughout the years. In 2001, many former workers still complained of psychic disorders, especially fatigue, headache, sleeping disturbances and/or internal disorders. Most of them also suffered from disturbances of lipid metabolism and/or nervous system lesions. The objective of this study was to investigate the incidence of disorders related to the occupational exposure to 2,3,7,8-TCDD. Nervous system disturbances were examined in a separate study (Lukaš et al. 2001).

### Methods

Subjects and sampling

All 12 former 2,3,7,8-TCDD workers were men, average age 56.8 (SD ± 2.1) years. Their exposure to 2,3,7,8-TCDD lasted from 10 days to 23 months. Nine of them were smokers (75%), ten drank alcohol every day (83%), and eight consumed at least one cup of coffee a day (67%). Examination was performed in accordance with the Declaration of Helsinki ethical standards and consisted of the following examinations:

1. **Internal and ophthalmological examinations and biochemical analysis of blood and urine.**
2. **Ultrasound examination** by Hewlett Packard Sonos 2000 system, always with a linear 7.5-MHz probe and Doppler-based evaluation of blood flow rates allowing colour coding of blood flow. We examined the whole extracranial bed without the knowledge of previous findings, to avoid the influence of previous evaluations. The intima-media thickness (IMT) (between two parallel lines on the inner side of the artery) was measured by visual assessment in the far wall of cranial (distal) segments of the common carotid artery, not in the bulb, in the longitudinal plane in three projections (directions) – anterolateral, lateral and posterolateral. The IMT was measured on repeated occasions at sites where it seemed to be the thickest. The three highest values of IMT on the left common carotid artery were used to calculate the average value.

3. **Neuropsychological examination with the following tests:**
   1. The Halstead-Reitan neuropsychological battery (HRNB) (Reitan and Wolfson 1993), a comprehensive set of neuropsychological measures, extensively used for clinical evaluation of brain behaviour relationships.
   2. The Wechsler adult intelligence scale-revised (WAIS-R) with six verbal and five performance subtests, which yields a verbal, performance and full scale IQ.
   3. The Wechsler memory scale (WMS) form I (Preiss et al. 1993), measuring remote and immediate recall, with transformation into age-corrected memory quotient.
   4. The Stroop colour word-test, a general test of cognitive efficiency on the basis of assessments of verbal and reading fluency and interference effects of colours and words.
   5. The Benton test of visual memory (Benton 1974), a test of visual retention, spatial memory, visual construction and visual form discrimination.
   6. The Seashore tonal memory test (Dodrill and Dikmen 1978), a short test that differentiates normal from neurologically impaired persons.
   7. Two original Czech questionnaires on neuroticism (N5, ZIS) and one original Czech questionnaire of subjective memory problems (Raisel et al. 1986).
   8. The computer program 'Train the Brain' (Sermon), Farmitalia Carlo Erba, a short measure of reaction time, memory of frequency of events and their sequence, spatial memory, and associative memory.

Statistics

For the statistical analysis, the statistical package SPSS for Windows was used. The association between two quantitative variables was measured by Spearman’s correlation coefficient. To measure the association between two categorical variables, we used the Fisher exact test. To compare proportions in paired data, we used the McNemara test. To compare means of quantitative variables in different groups, we used Student’s two-sample t-test or one-way ANOVA with Duncan’s test of multiple comparison. A value of $P < 0.05$ was considered as statistically significant.

### Results

Chloracne was seen as the first sign of intoxication and was acknowledged as an occupational disease. The year of notification and duration of this disease in 12 subjects are shown in Table 1. Plasma 2,3,7,8-TCDD levels 30 years after exposure (in 1996) are presented consecutively in descending order. Only subjects 1 and 3 still presented with chloracne. In subject 1, recurrent deep inflammation of cysts requires surgical interventions several times a year. A statistically significant difference in the level of 2,3,7,8-TCDD in plasma between the groups of patients with and without chloracne in 2001 was found ($P = 0.03$).

In Table 2 blood cholesterol, triglycerides, cardiovascular diseases and present hypolipidaemic therapy