Chronic health impairment due to 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin exposure

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Abstract

OBJECTIVES: The aim of this study, performed in 2008, was to evaluate the consequences of severe occupational intoxication with 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) that occurred during production of the herbicide trichlorophenoxyacetic acid in the period 1965-1968.

DESIGN: Examination of 11 men, mean age 64.4±1.5 years, included: internal and neurological examination, eye fundus examination, TCDD in plasma, thyroid-stimulating hormone (TSH), testosterone and serum lipids, ultrasonography of the carotid artery, nerve conduction study (NCS), electroencephalography (EEG), visual evoked potential (VEP), Lanthony test of acquired visual impairment, single photon emission computer tomography (SPECT) of the brain, neuropsychological examination and carbohydrate-deficient transferrin (CDT), a marker of chronic ethanol intake.

RESULTS: Mean TCDD level in 2008 was still 274.0±181.2 pg/g blood lipids (reference level is 2-3 pg/g). All (100%) patients had residues of chloracne/chloracne consequences, atherosclerotic changes on the eye fundus and plaques in the carotid arteries. Progression of intima-media thickness (IMT) from a mean of 0.84±0.14 mm in 2003 to 1.09±0.18 mm in 2008 was observed. Ninety-one per cents of patients had impairment in SPECT of the brain; and 55% of patients had hyperfixation of the perfusion indicator as a measure of increased neuronal activity. Additionally, 91 % of patients were treated for hyperlipidaemia, 73 % for hypertension, 55 % for diabetes type 2, 45 % for ischemic heart disease, and 36 % for psychological disorders. The Lanthony test demonstrated acquired dyschromatopsia in 80 % of patients. Mean colour confusion index (CCI) was 1.438, which indicates impairment since 2003, when the index was 1.302. CDT was in the normal range and did not correlate with CCI. Neuropsychological status appeared stabilized in all 8 patients examined, with impairment in one or more parameter (memory, attention, verbal fluency, psychomotor speed, motorics) in comparison

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to the norm.

CONCLUSION: Forty years after intoxication, the blood level of TCDD is still 100 times higher than in the general population. Other PCDD/Fs were not elevated. A high percentage of subjects suffer from neurological and vascular disorders. No association of alcohol consumption with neurological impairment was seen, and the highly significant correlation between CCI and TCDD blood concentration suggests that acquired colour impairment was associated with TCDD but not with alcohol consumption. IMT significantly increased during past 5 years. The patients obviously need complex treatment, including intense hypolipidaemic and antidepressant therapy.

Abbreviations & units

BDI	– Beck Depression Inventory
BMI	– body mass– index
CDT	– carbohydrate-deficient transferrin
EEG	– electroencephalography
GDS	– Geriatric Depression Scale
HRGC/HRMS	– high resolution gas chromatography/high resolution mass spectrometry
HRNB	– Halstead-Reitan Neuropsychological Battery
IMT	– intima media thickness
NCS	– nerve conduction study
PCDD/F	– polychlorinated dibenzo-*p*-dioxins and dibenzofurans
SPECT	– single-photon emission computer tomography
TCDD	– 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin
TEQ	– toxic equivalent (to TCDD concentration)
TSH	– thyroid-stimulating hormone
VEP	– visual evoked potentials
WAIS-R	– Wechsler Adult Intelligence Scale-Revised
WMS	– Wechsler Memory Scale

INTRODUCTION

The chemical 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) with an 8-year plasma half-life, chronically affects the cardiovascular system and the central nervous system. In addition it belongs to a family of persistent organic pollutants that can cause endocrine dismodulation, cancer and other disorders (Portier, 2002; Krcmery & Kalavsky, 2007; Kovarova & Svobodova, 2008). In TCDD-exposed subjects, an increased risk of lung cancer, soft-tissue sarcoma and non-Hodgkin lymphoma has been found; overall, the strongest evidence for the carcinogenicity of TCDD was for all cancers combined (IARC, 1997). The aim of this study was to evaluate the delayed consequences of severe chronic intoxication with dioxin during the production of the herbicide trichlorophenoxyacetic acid in the period 1965–1968.

MATERIAL AND METHODS

Eleven subjects (out of about 80 in 1965) were examined in a follow-up visit with the focus on internal, biochemical, neurological and neuropsychological changes caused by TCDD. The results were compared with the findings from previous examinations (Pelclova *et al.* 2001, 2002, 2007; Urban *et al.* 2007). The patients gave their written consent for the examination, which was a part of their follow-up visit concerning their occupational disease. Only eight patients agreed to a neuropsychological examination.

Examination included: body mass index (BMI) and body fat measured by bioelectrical impedance analysis, eye fundus examination, serum lipids, thyroid-stimulating hormone (TSH), testosterone and carbohydrate-deficient transferrin (CDT, index of a long-term alcohol consumption) assessment in the blood. The TCDD level was measured by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). Toxic equivalent to TCDD concentration (TEQ) was calculated according to Van den Berg *et al.* (1998).

Duplex Doppler ultrasonography was performed by a linear probe 7.5–11 MHz of the Phillips SONOS 5500 device. The examinations included the standard examination of the neck arteries with the attempt to exclude a significant stenosis. Then, intima media thickness (IMT) of the common carotid artery was measured by calipers within 10 mm proximal to the bifurcations, on the deeper wall. Mean value of 5 measurements was recorded on both sides as reported in detail by Holaj *et al.* (2003).

Neurological status was evaluated through a standardized clinical examination. Diminished sensation to touch and pain in a stocking-glove distribution, diminished vibration sense, bilateral loss of ankle and/or knee jerks, or bilateral diminished deep tendon reflexes in the lower as compared to the upper extremities were considered to be clinical signs of peripheral neuropathy.

Nerve conduction study (NCS): Conduction velocity was measured in motor and sensory fibers of the median, ulnar, tibial, and sural nerves using a Neuromatic 2000 (Dantec) device; *electroencephalography (EEG)* was recorded with a 21-channel digital machine (Walter-Graphtek) on conscious patients in a supine position with eyes closed. *Visual evoked potentials (VEP)* were examined with a 2-channel portable Keypoint system (Dantec). *The Lanthony 15-Hue desaturated panel* (L-D15-d) was used to test colour discrimination, because toxic acquired dyschromatopsias usually impair blue-yellow discrimination and cause type III dyschromatopsia (Verriest, 1963). Details of the methods are given in Urban *et al.* (2007).

Perfusion brain SPECT was performed after an intravenous injection of 600 MBq ^{99m}Tc-HMPAO (exametazim, CERETEC^{TM}) with a GE Infinia 2-head rotating gamma camera using a high-resolution parallel hole collimators in 128 × 128 matrices with 180 increments over 360°. The counting was set to the limits 80–100,000 imp/picture for 30–40 minutes of acquisition time. Transaxial images were obtained by the filtered back-projection method. Pre- and postfilter Butterworth power 10, cutoff 0.5. Data was recalculated to produce transaxial slices parallel to the orbitomeatal

line, and sagittal and coronal slices were calculated from the original transaxial images. Assessment of regional cerebral blood flow was performed visually and semiquantitatively (with the voxel-b-voxel method, NeuroGam™) by quantitative comparison with the activity of the cerebellum, with identical structures of the contralateral hemisphere and identical structures of the reference group of the males with comparable age. Anatomical identification was based on the Talairach Atlas.

Neuropsychological examination included:
1) Trail Making Test and Finger Tapping Test from the Halstead-Reitan Neuropsychological Battery-HRNB (Reitan & Wolfson, 1993);
2) Wechsler Adult Intelligence Scale-Revised – WAIS-R (Wechsler, 1981), subtests Digit Span, Similarities, Picture Completion, Block Design;
3) Wechsler Memory Scale (WMS) Form I (Wechsler, 1945);
4) Stroop Colour-Word Test (Treverry *et al.* 1994);
5) Verbal Fluency Test (Benton *et al.* 1983);
6) Beck Depression Inventory II – BDI-II (Beck *et al.* 1996);
7) Geriatric Depression Scale – GDS (Yesavage *et al.* 1983); and
8) two original Czech neuroticism questionnaires (N5, ZIS).

Statistical Analysis: The t-test, Spearman's correlation coefficient (r_s), the ANOVA test, and the statistical F-test were used. Uncertainty in estimation of the results was expressed in the form of confidence bands, which were calculated with 95% probability.

Table 1: Results in TCDD-exposed patients in 2008

Parameter	Mean	Reference range	Units
TCDD± SD	274.0±181.2	2–3	pg/g blood lipids
TEQ PCDD/F± SD	335.7.±172.6	15–50	pg/g blood lipids
CDT± SD	2.46±0.66	0–2.6	%
IMT± SD	1.09 ±0.18	<0.8	mm
TSH± SD	1.52±0.52	0.37–5.00	mIU/l
Testosterone± SD	13.9±5.9	5.4–19.5	nmol/l
BMI± SD	29.7±5.1	20–24	-
Body fat± SD	30.85±7.53	18–22	%
Body fat± SD	29.32±12.45	18–26	kg

Table 2: Percentage of findings in 11 TCDD-exposed patients in 2008 (refs. Cífková et al. 2004, Hříbek M et al. 2003, Josse et al. 1987, Pollack and Brodie 1998, Vrablík and Štulc 2003)

	N	%	Prevalence in the male population (comparable age)
Residues after chloracne	11	100	0
Eye fundus abnormalities	11	100	15
Plaques in carotid arteries	11	100	40
Hyperlipoproteinaemia	10	91	72
Hypertension	8	73	66
Diabetes type 2	6	55	18
Ischaemic hearth disease	5	45	20

RESULTS

Dioxin levels were still high; the main congener was TCDD as can be seen in ***Table 1***. Other congeners were at the usual population levels. Clinical findings are presented in ***Table 2***. The occupational history of the patients shows, that also cleaning of the plant, occupation of bricklayer and maintenance man belonged to important sources of TCDD exposure (***Table 3***). IMT significantly increased from a mean of 0.84±0.14 mm in 2003 to 1.09±0.18 mm ($p<0.001$). Mean cholesterol and triglycerides during the follow-ups showed a decrease, as can be seen in ***Figures 1 and 2***; however, the average levels of triglycerides did not mutually differ ($p=0.66$) within the period 2001–2008. On the contrary, the average levels of cholesterol in serum exhibited a decreasing trend, and the ANOVA test proved that the average levels in the years investigated differ ($p<0.001$).

Accordingly, the number of patients on hypolipidaemic treatment increased from 6 to 10 subjects; however, 7 subjects still had levels exceeding the upper limit of the reference range. The number of patients treated for hypertension increased from 7 to 8, and similarly the number of diabetics from 4 to 6. Four patients were treated with oral antidiabetics, and the other two with a diabetic diet only. On the other hand, the number of patients treated for psychological disorders decreased from 6 to 4 subjects. Cytochrome P450 system has not been studied, however the mean levels of liver enzymes were in the normal range (data not shown).

In the *neurological examination* only 4 of 11 patients had normal neurological results. Electrophysiological investigation revealed mild polyneuropathy in 7 (64%) patients, and ulnar cubital mononeuropathy in one patient. Subjects with polyneuropathy had lower CDT (2.22%) than subjects without polyneuropathy (2.89%). Diabetes was present both in the patients with polyneuropathy (3×) and in patients without polyneuropathy (3×).

EEG was normal in 8 of the 11 patients. In one patient, the frequency of occipital alpha activity was relatively slow in comparison with the previous examination in 2003. Only one patient's results were abnormal showing episodes of generalized rhythmic theta activity. This finding was

Table 3: Data concerning occupational exposure and plasma level of 2,3,7,8-TCDD in 11 examined men

Patient No	Born	Occupational Exposure	Time of exposure	Duration of Exposure	2,3,7,8-TCDD
					pg/g plasma fat
1	1945	chemistry technician and cleaning of the plant	1967–68	10 months	756
2	1943	chemistry technician	1965–67	23 months	415
3	1944	leader of the shift in the production	1967–68	12 months	364
4	1944	worker in the production	1968	2 months	272
5	1945	maintenance man	1966–67	11 months	264
6	1940	maintenance man	1967	3 months	236
7	1944	bricklayer in the workshop	1967	10 days	185
8	1944	bricklayer in the workshop	1967	2 months	179
9	1946	maintenance man	1966–67	10 months	153
10	1944	worker in the production and cleaning of the plant	1968	10 days	137
11	1942	maintenance man	1966–68	15 months	53

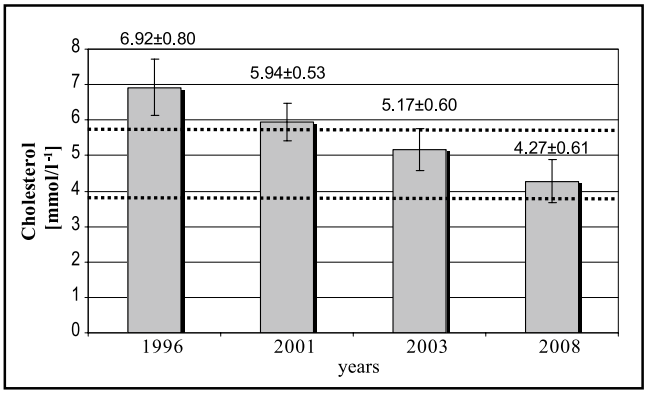

Figure 1: Mean serum cholesterol levels in the period 1996-2008 (reference range 3.83-5.80 mmol/l – the band between the dotted lines)

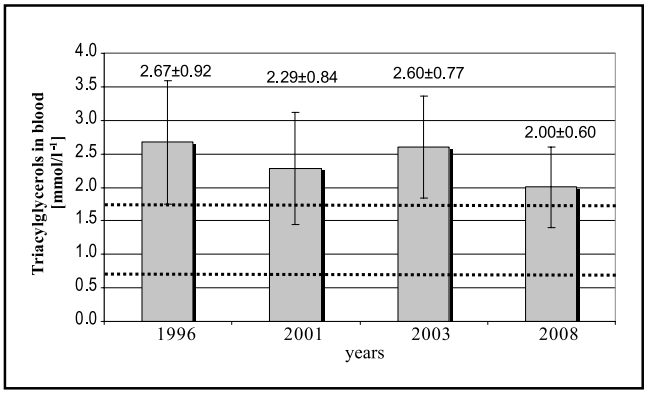

Figure 2: Mean serum triglycerides levels in the period 1991-2008 (reference range 0.68-1.69 mmol/l – the band between the dotted lines)

already present in 2003. There were no significant changes in EEG results over time.

VEP was normal in 3, and borderline in 4 of the 11 patients. The abnormal result in three of the patients was always compatible with visual acuity impairment. There was no significant change from 2003 to 2008.

The Lanthony D15d test was performed in all 11 patients; however, one patient had to be excluded due to congenital dyschromatopsia. Therefore, 10 patients were eventually evaluated. Eight patients showed results compatible with acquired type III dyschromatopsia according to Verriest (1963). In comparison with the previous examination, colour vision deteriorated in six patients, improved in four and showed no significant change in one patient. On a group level, the colour confusion index (CCI), i.e. the global measure of colour vision impairment, had slightly deteriorated from the mean value of 1.302 in 2003 to 1.438 in 2008; however, the change was not statistically significant (paired t-test: p=0.131). Spearman's rank correlation coefficient r_s of the correlation between TCDD blood concentration and CCI was 0.58, which is highly statistically significant (p=0.009). In contrast, the correlation between CCI and CDT was insignificant (r_s=0.36, p=0.13).

SPECT in the patients showed a strikingly higher percentage of findings with hyperfixation of the perfusion indicator as a measure of elevated neuronal activity in comparison with both the reference population and the SPECT examination in 2003. Three subjects had unilateral, and three patients had bilateral hyperfixation. On the other hand, four patients had bilaterally reduced perfusion in some brain regions. In ninety-one percent of the subjects was the SPECT finding evaluated as abnormal, comparing with the reference group. Unfortunately, the method previously used (with ^{99m}Tc-ECD (bicisate – Neurolite^{TM}) does not allow direct comparison.

Neuropsychological examinations in 8 patients did not find significant changes compared to the last examinations, and the status appeared stabilized with several impairments in comparison to the norm in all 8 individual cases in one or more parameters (memory,

attention, verbal fluency, psychomotor speed, motorics). In the presently discussed smaller group, no significant correlations of neuropsychological efficiency to TCDD levels were observed in contrast to the findings in 1996 (Pelclova *et al.* 2001) and 2003 (Urban *et al.* 2007). Now there was a trend towards lower memory performances with higher levels of TCDD (Memory Quotient, Wechsler Memory Scale, r=-0.611, p=0.108, Spearman's rank correlation coefficient).

During the past 5 years, two cancers were diagnosed in this group of patients. One patient died from thyroid cancer and one had a surgery for kidney cancer.

DISCUSSION

The results show that 40+ years after intoxication, the blood level of TCDD is still 100 times higher than in the general population. Other PCDD/Fs obviously did not play a role in this intoxication. The pathogenesis of damage due to TCDD is gradually elucidated by experimental data that have appeared especially in recent years (Arzuaga *et al.* 2007; Kopf *et al.* 2008; Nishiumi *et al.* 2008; Jung *et al.* 2009; Kim *et al.* 2009). Clinical findings reflect these results. Insufficiently controlled hyperlipidaemia and significantly increased IMT still appear to be problems in subjects with a high prevalence of ischemic heart disease, even if the mean levels of blood lipids have shown a significant decrease since 1991. The average levels of triglycerides in serum were still above the upper limit of the reference range and did not differ significantly in individual years. On the other hand, the levels of serum cholesterol in the subjects decreased with time and the average values have been within the reference ranges since 2003.

Among the patients with polyneuropathy, no association neither with diabetes nor higher CDT points to the probable influence of TCDD. In addition, the Lanthony test demonstrated acquired dyschromatopsia in a high proportion of patients, which showed a mild tendency to deterioration from 2003 to 2008. The highly significant correlation between CCI and TCDD blood concentration might imply a causal connection between colour vision impairment and exposure to TCDD.

SPECT showed a strikingly higher percentage of findings with hyperfixation of the perfusion indicator as a measure of elevated neuronal activity. To our knowledge, this is the first result of this kind, which needs to be followed up.

There were no significant findings in the EEG and VEP examinations. The neuropsychological examination yielded stable results, and the increased subjective complaints of the patients might be caused by their omitting of the treatment.

In vivo studies in experimental animals suggest that there is a cross-talk between aryl hydrocarbon receptor and steroid hormone receptor signal transduction pathways, including androgen and thyroid hormone receptors (Matthews *et al.* 2005). In our patients, however, both mean testosterone and TSH were in the normal range. On the other hand, the proportion of type 2 diabetes was rather high, in comparison with the population level; despite the fact that the BMI did not exceed the population average. Presented results are supporting the serious impact of TCDD exposure, documented by the long-term studies of the Joint Russo-Vietnamese Science and Technology Tropical Centre Hanoi on the consequences of chemical pollution of nature in Vietnam after the massive use of dioxin-containing defoliants in the late 1950s–1975, leading several decades later to a health loss in victims comprising 1–1.4 years of successful life for each 10 years lived (Rumak *et al.* 2009; Sofronov *et al.* 2002).

Unfortunately, no causal treatment to eliminate TCDD from the human body is available. Therefore, patients require continuous intense hypolipidaemic and neuropsychological treatment.

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