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Comparison of questionnaire data and analyzed dioxin concentrations as a measure of exposure in soft-tissue sarcoma studies

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HIGHLIGHTS

- Self-reported exposure to chemicals contaminated by dioxins suggested high odds ratios for soft- tissue sarcoma in a case-control study.
- Chemical analysis of dioxins in the same patients and controls indicated no risk.
- The results suggest that recall bias has confounded previous soft-tissue sarcoma studies.
- The role of the main chemical (chlorophenols or phenoxy acids) is not fully ruled out.

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ABSTRACT

Soft-tissue sarcoma is one of the few specific tumors thought to be caused by polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and specifically TCDD. Evidence is, however, based on questionnaire-based case-control studies, and on very few cancer cases in cohort studies at high occupational exposures to chlorophenols or chlorophenoxy acid herbicides with dioxin impurities. Recall bias has been suspected to influence the reporting of exposure, but this possibility has never been adequately put to test. In the present study 87 cancer patients and 308 controls answered a questionnaire asking their exposure to wood preservatives, fungicides and herbicides, and insecticides, and their PCDD/F concentrations were also measured. After matching for age and area 67–69 sarcoma patients and 153–156 controls were available for the study depending on the chemical group, 1–3 controls for each sarcoma patient. Sarcoma patients reported exposure to these chemicals significantly more often than controls did, odds ratios were 6.7 for wood preservatives ($p = 0.02$), 16 for fungicides and herbicides ($p = 0.01$), and 4.9 for insecticides ($p = 0.06$). There was no association, when the analysis was based on measured PCDD/F concentrations (odds ratios close to 1). Although it is not possible to exclude the role of the main chemical as the cause with certainty, the results indicate that recall bias is very likely in previous studies. Thus the causality between contaminant PCDD/Fs and soft tissue sarcoma cannot be considered proven.

1. Introduction

Dioxins (polychlorinated dibenzo-*p*-dioxins and dibenzofurans, PCDD/Fs), chlorophenols, and chlorophenoxy acid herbicides have been implicated in the etiology of soft tissue sarcoma as one of the few specific cancers identified (Hardell and Sandström, 1979; Kogevinas et al., 1997; IARC, 2012 Kogevinas et al., 1997; IARC, 2012); otherwise dioxins have been suggested to slightly increase the risk of all cancer combined (Kogevinas et al., 1997; Steenland et al., 1999; IARC, 1997, 2012). The carcinogenicity of chlorophenols

Abbreviations: CI, confidence interval; OR, odds ratio; PCBs, polychlorinated biphenyls; PCDD/Fs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; TCDD, 2,3,7,8-tetrachlorodibenzo -*p*-dioxin; WHO, World Health Organization; WHO-TEQ, toxic equivalencies according to WHO.

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and phenoxy acids was attributed to PCDD/F contaminants (IARC, 1997). The evidence on soft tissue sarcoma has been based largely on case-control studies using questionnaires to assess exposure (Cole et al., 2003) and on occupational cohort studies with very few cases of soft tissue sarcoma (Kogevinas et al., 1997; Steenland et al., 1999). No increased risk of soft-tissue sarcoma has been noted after accidents with high exposures to PCDD/Fs (Onozuka et al., 2009; Pesatori et al., 2009).

From the beginning of 1980s it has been suspected that there is a major possibility of recall bias in using questionnaire information in exposure assessment (Hardell, 1981), and no differences in PCDD/F concentrations were found between the exposed persons and controls (Nygren et al., 1986). However, as yet there has been no attempt to clarify this matter by comparing questionnaire data and actual dioxin analysis by investigating the same group of patients within the same study.

Scientifically sound and correct cancer risk estimates are preferable to ill-founded precautionary estimates, because these may lead to wrong priorities. Extreme control measures by authorities can also be directly harmful, e.g. by discouraging breast feeding (WHO, 2000) and decreasing fish consumption (Tuomisto et al., 2004b). Health benefits of these have been calculated greater than even pessimistically assessed risks of dioxins (Tuomisto, 2005).

We have previously published a large case-control study on the association between soft tissue sarcoma and PCDD/Fs indicating no increased risk associated with WHO-TEQ or individual congeners, e.g. TCDD (Tuomisto et al., 2004a). This study was not recognized by the IARC working group (IARC, 2012). In this study, subcutaneous fat samples were collected from 954 patients with soft tissue sarcomas or controls undergoing appendicitis operation, and PCDD/Fs were analyzed. Because of the long half-lives of PCDD/Fs, the analysis reflects the exposure over most of the lifetime. Patients also filled in a questionnaire asking a number of variables, including their dietary habits, weight history, and chemical exposure. This study material allows us to assess the magnitude of the recall bias that may exist, by studying in detail whether the observed associations between PCDD/F exposure and soft tissue sarcoma are similar regardless of whether the exposure is measured based on questionnaire data or the actual concentrations of PCDD/Fs in fat.

Three of the questions dealt with exposure to wood preservatives, agricultural fungicides and herbicides, and insecticides. The most common group of wood preservatives in past was chlorophenols which contain various PCDD/Fs as synthesis byproducts (Vartiainen et al., 1995a). Chlorophenols were implicated in soft-tissue sarcoma in several case-control studies (Hardell and Sandström, 1979; Eriksson et al., 1981 Eriksson et al., 1981). Pesticides are more diverse group of chemicals, among them PCDD/F impurities are found in chlorophenoxy acid herbicides (IARC, 1997) which were also implicated in soft tissue sarcoma in case-control studies (Hardell and Sandström, 1979; Hardell and Eriksson, 1988Hardell and Sandström, 1979; Hardell and Eriksson, 1988). Subsequently these authors assumed that the associations were possibly due to PCDD/F impurities (Hardell and Eriksson, 1988). The initial odds ratios were five to six (Hardell and Sandström, 1979), and in later studies around two to three (Hardell and Eriksson, 1988; Hardell et al., 1995 Hardell et al., 1995). Several other groups did not find an elevated risk (cf. IARC, 1997).

In the present study we compare the odds ratios based on positive answers on exposure to these groups of chemicals with the odds ratios based on dioxin concentrations in the patients and controls.

2. Material and methods

2.1. Soft tissue sarcoma patients and referents

A detailed description of sample collection and PCDD/F analysis has been given in the previous papers (Tuomisto et al., 2004a; Kiviranta et al., 2005).

Briefly, sarcoma patients attended the University hospitals of Helsinki, Kuopio, Turku and Tampere. All patients over 15 years of age operated for soft tissue sarcoma between June 1997 and December 1999 were eligible as cases. Patients over 15 years of age and operated due to appendicitis in any study hospital from the same catchment area were eligible as controls. Informed consent was obtained from all patients in writing before the operation, and the study was duly approved by the ethics committees. The total number of patients recruited was 972, and after exclusion of some patients for technical reasons (e.g. too small sample volume, see Tuomisto et al., 2004a), data on 954 patients (148 cases and 806 controls) were available.

A subcutaneous fat sample obtained during an appendectomy or sarcoma operation was analyzed for 17 PCDD/F congeners using gas chromatography – mass spectrometry (Vartiainen et al., 1997) at the Laboratory of Chemistry of the National Public Health Institute of Finland (currently Chemicals and Health Unit of the

Table 1
Number of patients reporting exposure to pesticides and wood preservatives against soft-tissue sarcoma status (case/control) available for matched analyses.

Reported exposure		Numbers of soft-tissue sarcoma cases and controls in differently exposed groups	
		Case	Control
Wood preservatives	Yes	8	2
	No	61	152
	Total	69	154
Fungicides and herbicides	Yes	7	4
	No	60	151
	Total	67	155
Insecticides	Yes	6	2
	No	61	151
	Total	67	153
Any of the three chemical groups	Yes	15	7
	No	54	149
	Total	69	156

National Institute for Health and Welfare), which is an accredited testing laboratory (Centre for Metrology and Accreditation code T077, EN ISO/IEC 17025). The scope of the accreditation covers the determination of PCDD/Fs in human samples. The laboratory participates regularly in interlaboratory exercises. All analytical work was performed blind and strict quality assurance measures were undertaken.

For the purposes of the present study, all patients, whose PCDD/Fs were analyzed, and who had adequately filled in the questionnaire answering the questions on exposure to wood preservatives, agricultural fungicides and herbicides, and/or insecticides were selected, consisting of 87 cancer patients and 308 controls. PCDD/F concentrations were as WHO-TEQ (van den Berg et al., 2006).

2.2. Statistical analysis

The association between exposure to PCDD/Fs and soft-tissue sarcoma was studied using conditional logistic regression.

Among the soft-tissue sarcoma cases and controls meeting the inclusion criteria, ORs were calculated for 67–69 cases and 153–156 controls (depending on the group, see Table 1) available after matching for age and area. There were 1 to 3 controls for each soft tissue sarcoma case. Age matching is important, because dioxin concentrations increase in non-linear fashion with age (Tuomisto et al., 2004a). Area was matched, because a major part of dioxin exposure in Finland is from Baltic fish, the consumption of which was expected to vary remarkably between the coastal and inland areas.

All statistical analyses were performed with R version 3.1.2., and the code is openly available at http://en.opasnet.org/w/KTL_Sarcoma_study#Self-reported_chemical_exposure

3. Results

Around ten percent of sarcoma patients reported to have been exposed to each individual group of these chemicals, while one to two percent of the controls reported exposure to each chemical group (Table 1).

Odds ratios were quite high for all chemical groups and statistically significant for wood preservatives as well as for fungicides and herbicides when calculated on the basis of queries, but close to 1 when based on chemical analysis (Table 2).

4. Discussion

Our results severely question the reliability of memory based data to indicate exposure in dioxin cancer studies. When questionnaires were used as a measure of exposure, wood preservatives, as well as fungicides and herbicides, significant risk factors appeared for soft tissue sarcoma in a matched pair analysis (OR 6.7 and 16, respectively). However, when exposure assessment was based on the actual levels of PCDD/Fs in the same patients, no association was found (OR close to 1).

Theoretically, there are two possibilities that can explain this finding. Either a recall bias, well known in epidemiology, is involved, and cancer patients are more prone to recall their exposure than the referents, or the association is not with contaminant dioxins but with the main chemical. Both possibilities cause concern as to dioxin risk assessment, since e.g. the IARC monograph 1997 states: “The effects of 2,3,7,8-TCDD and those of the products in which it was found cannot be separated in most epidemiological studies; however, the focus here is on the contaminant.” (IARC, 1997, p. 336). In other words, IARC working group was not sure which is the true cause, but because it was working on TCDD, this was implicated.

There are plausible arguments for either possibility. Most of the assessments of cancer risks of dioxins are based on epidemiological studies on mixed exposures including chlorophenols and/or phenoxy acids, and dioxins have been measured in very few studies. In field studies (e.g. workers spraying phenoxy herbicides) dioxin levels in workers have not been very high, usually close to those in general population (Nygren et al., 1986; Smith et al., 1992; Hardell et al., 1995; Pearce and McLean, 2005), and well within the range of concentrations in our original sarcoma study (Tuomisto et al., 2004a). It is noteworthy that chlorophenols and phenoxy acids are absorbed dermally (Jorens and Schepens, 1993; Knopp, 1994) while dioxins are absorbed very poorly (van den Berg et al., 1994).

In industrial worker studies with high dioxin concentrations the chlorophenol exposures needed to cause the measured PCDD/F levels are extremely high (Tuomisto and Tuomisto, 2012). The same applies to accidents with PCBs as the main chemical (Onozuka et al., 2009).

Furthermore, in a ground water contamination episode, a population of 3500 people was exposed to chlorophenols but not dioxins in drinking water for at least 15 years, and increases of soft-

Table 2
Odds ratios for soft-tissue sarcoma. Four separate models were run, where questionnaire information and PCDD/F TEQ were entered in the same model (i.e. mutually adjusted). There were 1–3 controls for each sarcoma patient and the association was studied using conditional logistic regression. Age and area were matched, and sex was controlled as a confounder. TEQ results are given per 20 pg/g fat which is close to the interquartile range in this population.

Predictor	OR for soft-tissue sarcoma	95%CI	N	p
Reported exposure to wood preservatives	6.7	1.4–33	223	0.02
PCDD/F TEQ	0.96	0.60–1.54		0.88
Reported exposure to fungicides and herbicides	16	1.9–138	222	0.01
PCDD/F TEQ	0.87	0.50–1.51		0.63
Reported exposure to insecticides	4.9	0.92–26	220	0.06
PCDD/F TEQ	0.90	0.54–1.49		0.67
Reported exposure to any of the three chemical groups	7.0	2.2–22	225	<0.001
PCDD/F TEQ	0.84	0.49–1.42		0.51

tissue sarcoma and non-Hodgkin lymphoma were detected (Lampi et al., 1990, 1992a, 1992b; Vartiainen et al., 1995a, 1995b). The IARC assessment noted the chlorophenol exposure study, but not the studies showing there was no simultaneous PCDD/F exposure (IARC 1997, p. 75). The numbers of cancers were low, but this has been the case in the few positive studies suggesting PCDD/F involvement in soft-tissue sarcoma (Kogevinas et al., 1997; Steenland et al., 1999).

Increased odds ratios regardless of the chemical group may challenge the main chemical hypothesis, although the OR of insecticides was not significantly elevated. Therefore, this study cannot differentiate with certainty between the two possible explanations, but recall bias seems more important and likely. In either case it clearly challenges the interpretation of case-control studies indicating that soft-tissue sarcoma would be caused by PCDD/Fs, and the small proportions of sarcoma patients exposed in the studied population show that none of these chemicals can be a major cause of soft-tissue sarcoma.

Conclusion: Considering all of the available data without overemphasis on positive results, one may state that dioxins may be at most possible carcinogens in the causation of soft-tissue sarcoma at high industrial doses, but not that they would be proven human carcinogens (for ref. see the minireview of Tuomisto and Tuomisto, 2012).

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