Polychlorinated dibenzo-*p*-dioxins and the human immune
system: 4. Studies on two Spanish families with increased
body burdens of highly chlorinated PCDDs

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Abstract

The consequences of exposure of people to highly chlorinated polychlorodibenzo-p-dioxins (PCDDs) are much less known than those of TCDD. We report on levels of PCDDs (and PCDFs) in 13 members of two families poisoned by contaminated cooking oil. Originally, all persons displayed chloracne as an early symptom. Persisting hexa- and higher chlorinated PCDDs could be analysed many years after exposure. Highest values found in blood lipids were: OCDD 660,000 pg/g; HpCDD 58,000 pg/g; HxCDDs: 3,500 pg/g. None of the participants exhibited increased TCDD levels at the time of study. During a period of 6 years, HpCDD and OCDD disappeared from the blood lipids much faster in persons exposed as children or young adults, than from lipids of their parents. Surface receptors on blood lymphocytes of the members of the two families and the proliferative capacity of these blood cells in the presence of typical stimulants were analysed. Even in family members with the highest body burdens of hexa- to octachlorinated PCDDs we could not detect pronounced changes from a reference population with respect to the immunological markers. Minor deviations of levels of some receptors in a few, but not all, highly exposed persons suggested a similar trend to those reported in previous studies of persons with body burdens of $\geq 3,000$ pg TCDD/g blood lipids. An increase in the number of total blood lymphocytes in some subjects exposed as children may have similarity with highly TCDD-exposed children in Seveso.

Key words: Octachlorodibenzo-*p*-dioxin, heptachlorodibenzo-*p*-dioxin, dioxins, immune system, humans.

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Abbreviations: PCDDs, polychlorinated dibenzo-*p*-dioxins; HxCDD, hexachloro-DD; PCDFs, polychlorinated dibenzofurans; HpCDD, heptachloro-DD; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; OCDD, octachloro-DD; TEF, toxic equivalency factor; TEQ, toxic equivalent quotient; SD, standard deviation.

1. Introduction

The persistence of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in the environment, food and people (Geyer et al., 2002; Kahn et al., 1988; Needham et al., 1994; Needham et al., 1999; Neuberger et al., 1999; Neubert et al., 1990; Tohyama, 2002) continues to be of concern, but it has proven difficult to ascertain the true risks for human health with respect to the various congeners and mixtures of these contaminants (called here collectively, dioxins), especially at a low dose range (Neubert, 1997/98). Originally, most of the concern was based on experimental evidence showing an excessive toxicity, including some carcinogenicity, of the most potent chemical, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), in the liver of rodents, and in humans on the induction of chloracne (Baccarelli et al., 2005), skin lesions for which a special mouse strain may be a model (Panteleyev et al., 1997). Initially, both experimental as well as human data were from relatively high exposures (Pohjanvirta and Tuomisto, 1994). Despite considerable advances in molecular approaches, the exact mechanisms of many of the experimental observations have yet to be fully explained. In more recent years, particular attention has been paid to effects occurring at much lower exposures, especially those of reproductive and brain development, the immune system, and hormone-mediated effects such as thyroid homeostasis (Birnbaum and Tuomisto, 2000; Tohyama, 2002). Human risk assessment and hazard minimization is hampered by considerable species differences with respect to susceptibility and organotropy. Highly exposed human subpopulations have become rare, due to considerable reduction of emissions in many countries.

TCDD is known to cause immunotoxic effects in some rodent species (Burleson et al., 1996; Greenlee et al., 1985; Holsapple et al., 1991; Kerkvliet, 1995; Vos et al., 1991), and some immunological components respond to very low doses in non-human primates, i.e. within the low nanogram range (Neubert et al., 1991; Neubert et al., 1992b; Neubert et al., 1992c; Neubert et al., 1993b; Neubert et al., 1994a; Neubert et al., 1994b; Neubert et al., 1995). Some of the effects (e.g. thymus involution) are reversible when the pollutant has disappeared from the target (van Loveren et al., 1991). Species differences in the outcome are common, and the immune system of rats was reported to be much less vulnerable than that of mice (Smialowicz et al., 1994).

From the limited number of studies, the susceptibility of components and functions of the immune system of humans with TCDD body burdens has so far appeared minimal, compared to mice and monkeys. Volunteers with moderately increased body burdens were mainly studied, but in a few investigations persons with rather high exposure were included in the evaluations (Neubert, 1997/98; Neubert et al., 1993a; Neubert et al., 1994b; Neubert et al., 1994/5; Neubert et al., 1999b; Neubert et al., 2000; Zober et al., 1992). Conclusions are hampered by the rather small number of subjects in some studies, and results (Tonn et al., 1996) that could not be reproduced in other populations. Far less is known about the relative toxicities of the other PCDDs and PCDFs in humans. There are suggestions of immunological changes in children exposed to environmental PCDDs *in utero* and by breast-feeding (Nagayama et al., 1998; ten Tusscher et al., 2003; Weisglas-Kuperus et al., 2000; Weisglas-Kuperus et al., 2004), but at present the results are inconclusive, and the medical significance of the data is unknown. While studies on immunological components and functions have been performed on volunteers exposed exclusively to TCDD from the Seveso area (Neubert et al., 1999a; Neubert et al., 1999b; Neubert et al., 2002) and on workers with PCDD/PCDF exposure predominantly to this contaminant, to our knowledge no data are available specifically on people exposed to low body burdens of TCDD but with high burdens of hexa- to octachlorinated dibenzo-*p*-dioxins.

In this paper we report on data obtained from two Spanish families exposed by accident to cooking oil greatly contaminated with highly chlorinated dioxins. The poisoning scenario and clinical data for Family 1 were reported previously (Hansson et al., 1995b; Rappe et al., 1998; Rodriguez-Pichardo and Camacho, 1990; Rodriguez-Pichardo et al., 1991). The existence of Family 2 has only been briefly reported before (Rappe et al., 1998). Several of these persons exhibited highly elevated dioxin blood levels, predominantly hexachlorodibenzo-*p*-dioxins and higher chlorinated congeners, even after a period of 14 years. Therefore, it was of interest to evaluate whether exposure to predominantly HxCDD and higher chlorinated PCDDs as well as HpCDF causes a qualitatively and quantitatively different response on immunological components and functions than TCDD, and whether immunological variables may serve as sensitive biomarkers of such a dioxin exposure. These

immunological end points have been reported to be extremely sensitive to the action of various dioxins in non-human primates (Neubert et al., 1992b).

2. Methodology

2.1. Families

Family 1 consisted of a father (age 42), mother (age 39), 3 daughters and 3 sons that presented to the clinic in 1982 with varying degrees of chloracne ranging from severe to mild. A further son was born a few months after the family were first seen (Rodriguez-Pichardo and Camacho, 1990; Rodriguez-Pichardo et al., 1991). Blood samples from the most severe cases originally, were combined in 1987 and together with the contaminated olive oil analysed for PCDDs and PCDFs. Symptoms of chloracne had disappeared in all but the father after one year. The father also suffered from severe respiratory problems at the time of presentation and the youngest son developed convulsions soon after birth which did not reoccur after his mother stopped breast feeding. In early years his growth was apparently retarded (Rodriguez-Pichardo et al., 1991). Oil was consumed by use in cooking and as a salad dressing. Family 2 presented in 1990 with a mild degree of chloracne, apparently also poisoned with contaminated cooking oil in a similar, but unrelated, incident to Family 1. Exactly how the two olive oil batches became contaminated with PCDDs and PCDFs has never been fully resolved but probably involved reuse of plastic containers that had contained chemicals for pesticide preparation or other purposes. Individual blood samples from both families were collected and examined in 1990 and 1996 for analyses of PCDDs and PCDFs, and for immunological assessment of blood in 1996. Up to 1996 health problems other than chloracne and the initial observations of the father and youngest son of Family 1 described above, could not be ascribed categorically to consumption of the contaminated oils. Contact with Family 1 is now only occasional and we have no evidence of any related illnesses. Family 2 has been monitored regularly with no evidence of any unusual findings other than those of higher blood lipids in the parents which might be expected with age.

2.2. Reference ranges

Although data on human PCDD/PCDF body burdens of the general Spanish population were not available to us, values from large German data bases of the period of our investigations (Beck et al., 1991; Papke et al., 1996; Pöpke et al., 1994;

Päpke et al., 1995) allowed comparison on the extent of the high exposure to PCDDs of the two Spanish families.

For the investigation of the immunological components and functions we decided, in the absence of a true large control group, to use volunteers participating in other parts of an EC project investigating people exposed to dioxins at Seveso, Italy, as a reference group (Neubert et al., 1999b). This large number of controls had the distinct advantage that the controls could be age-matched and several of them, but not all, had undergone quantification of the PCDD-body burdens. The disadvantage was that this reference group was not from the same European country. For evaluating age criteria a group of 39 older reference persons was selected (48-65 years of age) and 44 younger persons (18-32 years of age), out of a data base of 255 subjects. Additionally, four staff persons from the hospital where the blood samples were drawn volunteered to provide local comparisons.

2.3. Blood sampling and handling

All blood samples for immune responses were drawn between 8 and 9 am at the Department of Dermatology, Faculty of Medicine, Sevilla, Spain. The blood was transferred without delay from the syringe into 4-ml-tubes (Greiner, Nürtingen, FRG) containing approximately 50 IU heparin (stabilisatorfrei, Seromed) and gently mixed. To protect samples destined for functional analyses they were immediately diluted with RPMI 1640 medium supplemented with 5% fetal calf serum (1:1 v/v) before subsequent transport by plane from Spain to Berlin, where they arrived < 8 hours. This time limit was set from previous experience when it was found that samples that had been transported for longer periods were no longer of use in stimulation tests. A complete hematological investigation was performed for each of the samples using a Sysmex F-800 electronic cell counter (Digitana, TOA Medical Electronics, Hamburg, FRG), and the data were supplemented and cross-checked by differential blood smear cell counts.

2.4. Analysis of PCDDs and PCDFs

Blood for the analysis of PCDDs and PCDFs (up to 200 ml) was withdrawn subsequent to that taken for immune responses. The analyses of the oil samples presumed to have poisoned Families 1 and 2, the pooled serum sample in 1987 and the subsequent analysis of blood plasma from both families in 1990 and 1996 were by

high resolution gas chromatography-mass spectrometry (Hansson et al., 1995a; Kahn et al., 1988; Nygren et al., 1988). Samples were cleaned-up using adsorbants before analysis and expressed relative to lipid content determined by gravimetric analysis. Results were within the WHO quality control range (Stephens et al., 1992).

2.5. Analyses of surface receptors

Analyses of the pattern of lymphocyte subpopulations were performed with whole blood. Samples of blood (50 µl) were reacted with a mixture of the specific monoclonal antibodies (mAbs) conjugated with peridinin chlorophyll, phycoerythrin or fluorescein-isothiocyanate in different combinations, generally as a tri-color assay, for 15 minutes at 4°C in the dark in the presence of 0.1% NaN₃. After incubation the samples were lysed (lysing solution lot #11, Dianova, Hamburg), fixed (fixing solution lot #11, Dianova, Hamburg), washed twice and additionally fixed with paraformaldehyde (1 %).

Surface receptors on the peripheral blood mononuclear cells were analyzed using a mono-laser (488 nm) flow cytometer (FACScan, Becton Dickinson, Heidelberg, FRG). All data were acquired as listmode data with subsequent gating performed individually on either the lymphocyte, monocyte or granulocyte fractions respectively, as defined by their characteristic forward and side scatter parameters. To distinguish between subpopulations on the basis of high and low receptor densities, calibrations with fluorescence conjugated latex beads bearing defined numbers of fluorescence molecules (Flow Cytometric Standards Corporation) were performed prior to sample evaluation. Analysis of the surface markers was performed with the follow mAbs against human lymphocyte epitopes: CD8 (T8), CDw29 (4B4), CD45RA (2H4), CD2 (T11), CD20 (B₁), CD56 (NKH1) from Coulter Electronics (Krefeld, FRG), and CD4 (anti-leu3a) from Becton Dickinson (Heidelberg, FRG).

The acquired data were analysed with the Winlist[®] software package (v. 3.0, Verity, Topsham, Me, USA) and the results were transferred to Microsoft's Excel[®] for further analyses. Statistical analyses were performed with Minitab[®] for Windows software or SPSS v. 7.5.

2.6. Analysis of the proliferative capacity of lymphocytes

Functional analyses were performed using whole blood diluted 1:8 v/v with RPMI 1640 culture medium (Seromed, Berlin) supplemented with 2.5% L-glutamine as well

as 5% heat-inactivated fetal calf serum. The diluted mixture was pipetted into 96 well round-bottom culture plates (Nunc Microwell Plates) and incubated for 6 days in an incubator (Heräus gas-incubator) at 37°C and 5% CO₂. Following the incubation period, 10µl of ³H-thymidine (Lot #215, activity 307 MBq/mg, Amersham) were pipetted into each well and incubation was continued for a further period of 6 hours. At the end of the incorporation period the plates were emptied and washed with a semi-automated cell harvester (Titertek, Flow Laboratories). The filter papers were dried at 40°C and the individual filter tags dispensed into polyethylene counting vials (Zinsser Analytische GmbH). 10ml of scintillation fluid (Quicksafe N #13077, Zinsser Analytic) were added and each sample was counted in a scintillation counter (Tri-Carb, Packard, Frankfurt) for a period of 5 minutes (Neubert et al., 1994/5). Stimulation of lymphocytes was performed with poke weed mitogen (PWM, Seromed, Berlin), phytohemagglutinine (PHA, Seromed, Berlin), concanavalin A (ConA, Seromed, Berlin), and mouse mAb against human CD3 (T3, Coulter Electronics, Krefeld FRG) (Neubert et al., 1999b).

2.7. Statistical evaluations

Because of the small number of participants, comparison was performed predominantly with the values of the outside reference group. Therefore, the data have a more prescriptive character. Multiple regression analyses of the principal confounders, the age of the subject, the smoking habits and the percentage or the number of lymphocytes/µl of blood was performed.

3. Results and Discussion

3.1. PCDD and PCDF body burdens

Analyses of blood levels of PCDDs and PCDFs in the families allowed comparisons of body burdens with a reference population, comparisons between members and changes with time, and correlation with some components of the immune system.

3.1.1. Exposure of Families 1 and 2, and individual PCDD and PCDF profiles

Repeated application of oil ingested by Family 1 to the ears of rabbits elicited a chloracne type response (A. Rodriguez-Pichardo, unpublished findings) but the dermal toxicity of the oil could not be demonstrated with a mouse model (Rodriguez-Pichardo et al., 1991). A porphyric response was observed in mice but porphyrin

levels in urine from patients on first visiting the clinic were not elevated. Preliminary analysis showed that the contaminated olive oil contained hexachlorobenzene and pentachlorophenol but that these did not account for its acnegeic toxicity. Further analysis of the oil showed the presence of considerable quantities of hepta- and octachlorodibenzo-*p*-dioxins (HpCDDs and OCDD) together with lesser amounts of the dibenzofuran analogues (Rodriguez-Pichardo et al., 1991) as summarized in Fig. 1A and 1B. It was particularly clear that the levels of TCDD and other less chlorinated congeners were very low. Pooled sera from the four originally most severely affected members of this family, father and three eldest children, analysed in 1987 (5 years after the reported ingestion) showed the presence of high levels of HpCDDs and OCDD, with low levels of TCDD, as the main constituents clearly reflecting the original oil (Fig. 1C and 1D). The contaminated olive oil responsible for the chloracne symptoms of Family 2 also contained mainly the higher chlorinated PCDDs and PCDFs with negligible amounts of TCDD. However, the absolute levels of all constituents were considerably less than in the oil used by Family 1 (Fig. 2A and 2B).

Blood samples from all 14 members of Families 1 and 2 were taken in 1990 and repeated in 1996 (except for one member of Family 2), and PCDDs/PCDFs contents were estimated for all samples. Plasma levels of "dioxins" are accepted as a marker of body burdens. Only small quantities of blood could be taken from the grandchildren of Family 1 in 1996.

For Family 1 the levels of PCDDs in plasma (1990) were considerably lower than those estimated previously in 1987 for the pooled serum (Table 1). For instance, pooled OCDD levels in 1987 were estimated as 936,000 pg/g lipid, but in 1990 ranged from 140,000-300,000 pg/g in those who contributed to the sample. Even so, PCDDs, mainly HpCDDs and OCDD, were 2-3 orders of magnitude higher than to be expected in controls or even in populations exposed at the workplace (Hansson et al., 1995a). Overall levels were highest in the parents and eldest children and lowest in the son born not long after first presentation of the family and presumably exposed *in utero* or in the short period on mother's milk. Unlike other episodes of accidental exposure to dioxins, TCDD levels were no greater than in control populations (Hansson et al., 1995a; Neuberger et al., 1999; Papke et al., 1996). PCDFs had fallen greater than PCDDs and contributed little to the body burden and so were not

included in Table 1. By 1996 PCDD levels had declined significantly in all children of Family 1 even for HpCDD and OCDD, for which human adult half-lives of approximately 100 years have been extrapolated (Geyer et al., 2002). Part of these declines may have been the consequence of increases in body mass and adipose tissue. The decline in OCDD was not observed with the parents and we could not reject the possibility of additional exposure by an unknown route. Two young children of the eldest daughter, who was exposed as an 11-year old, had markedly elevated levels of HpCDD and OCDD consistent with exposure *in utero* and during breast feeding.

Members of Family 2 had exhibited mild chloracne in comparison with the original symptoms of Family 1 and by 1996 acne could only be observed in the father. As with the first family, TCDD levels in plasma were very low, the major congeners both in 1990 and 1996 being HpCDD and OCDD (Table 2). Since less time had elapsed from initial exposure, levels of the PCDFs could still be detected especially of 1,2,3,4,6,7,8-HpCDF, but were far lower than the OCDDs reflecting initial oil concentrations (Fig. 2). Both of the two children that contributed blood in 1996, showed a marked fall in the level of HpCDDs, HpCDFs and OCDFs since 1990. With respect to OCDDs, body burdens of the parents in 1996 were greater than in 1990, at least based on one sample. This result is in accord with the data in Family 1, and cannot easily be explained by body weight changes.

3.1.2. Body burdens in adults versus young volunteers

Since blood samples were analysed in 1990 and in 1996, this enabled assessment of not only individual body burdens of PCDDs and PCDFs but also whether these changed during 6 years, a period of almost one half-life (about 7-8 years) estimated for TCDD (Needham et al., 1994; Pirkle et al., 1989). It is interesting that in both Spanish families the OCDD body burdens of the parents apparently increased during the 6 years from 1990 to 1996 (Tables 1 and 2), and the concentrations of HpCDD in blood (lipids) either stayed constant (Family 1) or (in Family 2) declined by 10-20%. This is consistent with the long half lives calculated for HpCDDs and OCDD (Geyer et al., 2002). In contrast, plasma PCDD levels in the daughters and sons declined between 1990 and 1996 to a far greater degree than might have been anticipated from estimates on the parents (Tables 1 and 2). This finding is in accord with reports on TCDD exposure in the Seveso area, Italy: elimination half-lives for TCDD were

reported to be much lower in children, when compared with the long half-life known for adults (Needham et al., 1997). Several factors, including increase in growth and total fat content, as well as a faster elimination may contribute to this phenomenon. For these reasons, the initial HpCDD/OCDD body burden in the small children originally may have been higher than at first anticipated.

Data shown in Tables 1 and 2 indicate that the body burdens in the volunteers exposed as children or youngsters declined within the 6 years between the two measurements much faster with regard to HpCDD than for OCDD. The percentages of the decline of body burdens from the levels in 1990 to those in 1996 were calculated as: HpCDD: 69% (F_B), 75% (M_C), 90% (M_F), 62% (F_G), 51% (F_J), 91% (M_H), 85% (M_K), 77% (F_M), 86% (M_L); mean decline $\pm$ SD 76 $\pm$ 14 %. OCDD: +18% (F_B), 0% (M_C), 82% (M_F), 38% (F_G), 7% (F_J), 69% (M_H), 54% (M_K), 47% (F_M), 79% (M_L); mean decline $\pm$ SD 54 $\pm$ 26 %.

An approximate estimation suggests that within the group of young volunteers the values in 1996 for HpCDD had declined to an average of about $\frac{1}{4}$ of the level in 1990, and the concentrations of OCDD had declined within the same period to an average of about $\frac{1}{2}$ (with a considerable inter-individual variability). These results are of relevance in itself, when considering an extrapolated half-life for OCDD in adults of about 100 years (Geyer et al., 2002).

3.1.3. Ratio of different PCDDs over a period of 6 years

Continuous exposure to a mixtures of PCDDs/PCDFs eventually results in a steady-state body burden depending on the half-lives of the various components. After exposure has ceased, the composition of the body burden must change with time reflecting the different elimination half-lives of the components. This has been shown for PCDDs in animal studies (Korner et al., 2002), and can be assumed for the Spanish families, unless all components of the mixture were eliminated from the adipose tissue at an extremely slow rate. A change in the composition of the body burdens is shown by the ratios between various components (Table 3). Within the members of one family these ratios were very similar. Since the composition of the exposure was probably identical, this provides an indication of the resulting inter-individual variability. No clear-cut difference could be recognized for the ratios shown between the parents and the children of Family 1, although the elimination

half-lives were shorter within the younger volunteers. There was a difference between older and younger participants in Family 2 (OCDD/ HpCDD). The inter-individual variability differed for the various ratios, and it was especially low for the ratio; 1,2,3,6,7,8-HxCDD/ 1,2,3,4,7,8-HxCDD. For the ratios evaluated, the 6-years difference was no larger than 2-3-fold.

3.1.4. Usefulness of TEFs with respect to human body burdens

TEFs for PCDDs and PCDFs are not designed to calculate human body burdens, unless reliable data on humans can be established which is extremely difficult. Thus present TEFs obtained experimentally are inevitably not completely suitable for human risk assessment (Neubert et al., 1992a; Neubert, 1997/98; van den Berg et al., 2006). The fact that the TEFs for some of the PCDD congeners have been modified repeatedly underlines the inherent uncertainties of the approach. For instance, TEF values for OCDD, the contaminant especially relevant in our study, has been criticised, and TEF-values of 0.0001 or even zero have been discussed (Sutter et al., 2006). However, present TEFs are pragmatic for assessing oral exposure or regulating industrial emissions. While TEQs (the TEQ is the sum of all PCDDs and PCDFs, each congener being multiplied with the corresponding TEF) are generally based on exposure data and doses and sometimes on *in vitro* data, there are results of studies in which typical effects of dioxin congeners *in vivo* are correlated with tissue concentrations providing information on the internal effective dose. Unfortunately, different TEQ-concentrations and different ratios of congeners occur with an identical exposure in different species and within different tissues of the same species.

In rats, hepatic cytochrome P4501A1-mediated activity induced by TCDD, HpCDD and OCDD has been correlated comparatively with tissue concentrations (Golor et al., 1992). The potency at the same tissue concentration of HpCDD was 20 to 25-times higher than that of OCDD. Since the concentration-effect curves were not strictly parallel, the potency of HpCDD was found to be fold less, when compared with TCDD (a factor of about 0.01 at the NOEL). The values established agree well with the presently suggested TEF-values. However, the effect was correlated with the PCDD concentration within the target tissue. The concentration ratio adipose tissue to liver varies greatly between species for the different congeners, and with time and other exposure conditions (Golor et al., 1992; Neubert et al., 1992a). Therefore,

extrapolation with the use of TEFs to possible body burdens existing within different human tissues remains speculative.

For our subjects, the contribution of the highly chlorinated PCDDs (from HxCDDs to OCDD) to the overall body burden would critically depend on the type of TEF applied (Table 4). With the problems of applying present TEFs to human body burdens, other estimates to the ones shown are possible. Our analytical data enabled the PCDD and PCDF composition of the contaminated food to be compared with the composition of the resulting body burdens. The best data are on the OCDD/ HpCDD ratio. With respect to the cooking oil associated with the Family 1 this ratio is >100 (72,000,000 ppt OCDD/ 400,000 HpCDD), which is well above the ratio found within the blood lipid of the volunteers in 1987, about 23, or in 1996 (Table 3).

3.2. Effects of higher chlorinated PCDDs on components of the immune system

To our knowledge there are no published data on immune components and function of adults with high body burdens of the highly chlorinated dioxin congeners. Although all the Spanish volunteers had initially exhibited varying degrees of chloracne, this had mostly resolved despite having levels of hexa-, hepta- and octa PCDDs, far higher than the general population. Since many immune factors are age dependent (Foerster et al., 1997), exposed parents were grouped as ‘older’, and a ‘younger’ group contained the daughters and sons. Because a full Spanish reference range for healthy volunteers was not available for these two subgroups, we used a reference population not especially exposed to dioxins (10-90 percentile) from a study performed at about the time in another partly EU-funded project in Seveso, Italy (Neubert et al., 1999b; Neubert et al., 2002). Comparison with the reference group was performed both for absolute values and the percentages of total lymphocytes (Tables 6a and b). In the few cases where statistically significant differences were found, these values were just outside the 80% range of the selected reference population. Data for local non-exposed Spanish adults were also obtained for comparison (Table 6c).

3.2.1. Components of the human immune system to be considered

Extrapolations between species in immune responses are hampered by clear-cut species differences and considerable variability exists in animal studies. However, monkeys have seemed the best model for humans studies on immunological

components and functions. Studies with the New World species (*Callithrix jacchus*) have the advantage that exactly the same methodology can be employed as used for humans, i.e. the antihuman mAbs employed cross-react (Neubert et al., 1999a; Neubert et al., 1999b), and can be utilized either for verifying the susceptibility in humans, or to suggest a lower sensitivity to these pollutants. Data on Old-World monkeys are also available (Hendrickx et al., 2002; Jonker and Slingerland, 1989). Alterations in the expression of some surface receptors on lymphocytes are the most sensitive endpoints after exposure of *Callithrix* to various PCDDs or to polybrominated congeners (Neubert et al., 1990; Neubert et al., 1991; Neubert et al., 1992b; Neubert et al., 1992c; Neubert et al., 1993b; Neubert et al., 1994b; Neubert et al., 1995). Single doses as low as 10 ng TCDD/kg body weight will reduce the number of CD4⁺CD29⁺ blood lymphocytes (equivalent to the human expression of CD4CD45R0-receptors [helper-inducer, memory-type cells]) clearly below the reference range in all treated monkeys. This decline is associated with a reduced expression of some integrin receptors (e.g. CD4⁺CD11a⁺ or CD4⁺CD18⁺), and with some other typical changes, including a reduced number of B-lymphocytes (CD20⁺ cells). Simultaneously, an increase in the number of CD4⁺CD45RA⁺ lymphocytes (naive-type cells) has been observed. The NOEL in *Callithrix jacchus* was estimated to be 1-3 ng TCDD/kg bw.

The changes in monkeys induced by TCDD and PCDFs suggested the immune parameters that could be evaluated in the Spanish families i.e. (a) decreased expression of the CD45R0-receptor on CD4⁺ cells (b) decreased expression of some integrin receptors (activation markers, CD29, CD11a, CD18), (c) increased expression of the CD45RA-receptor on CD4⁺ cells and the ratio: CD45R0⁺ / CD45RA⁺ as an indicator of these alterations, (d) decreased number of B-cells (CD20⁺) and (e) increased number of cytotoxic cells (CD8⁺CD56⁺) together with a decrease in suppressor cells (CD8⁺CD56⁻) and a change in the ratio: CD56⁺ / CD56⁻ / 57⁻. In addition, children highly exposed to TCDD (at Seveso) were reported to show a transient increase in total lymphocytes (Baccarelli et al., 2005).

From kinetic studies it is established that following a single dose of 1 ng TCDD/kg bw a concentration of 3 pg/g adipose tissue (3 ppt) results, in humans (Poiger and Schlatter, 1986). It is reasonable to assume that 10 ng/kg bw will lead to 30 pg/g lipid (Table 5). It may be assessed that in the monkeys a single dose of 10 ng TCDD/kg bw

may result in about 80 ppt (pg/g) in adipose tissue (Neubert, 1997/98; Neubert et al., 1999b). Some residents of the Seveso area (Italy) and clean-up workers had high body burdens between 100 and 5,000 ppt TCDD, and a slight increase in CD4⁺CD45R0⁺CD45RA⁻ cells (opposite to the expected effect of the monkey studies). A trend for a decrease in the number of CD4⁺CD45R0⁻CD45RA⁺ lymphocytes (naive-type cells) was found when the body burden exceeded 3,000 ppt. This trend was seen in men only and not in women, but almost all values stayed within the reference range (Neubert et al., 1999a; Neubert et al., 1999b; Neubert et al., 2002). Thus, there was a significant trend of a change, at high TCDD exposure levels only, but the deviation was not deemed sufficient to be medically relevant.

Although OCDD and HpCDD are expected to exhibit a lower potency than TCDD the true efficacy in humans remains unknown. Our investigations were aimed at showing evidence whether a body burden consisting exclusively of the higher chlorinated PCDDs would induce a more pronounced effect on lymphocyte surface receptors than TCDD. When compared with median values of a German reference population (Papke et al., 1996; Pöpke et al., 1994), the adult members with the highest body burdens of the two Spanish families exhibited the following greatly elevated mean concentrations of PCDDs in blood lipids: OCDD, Spanish ~ 500,000 ppt, reference ~300 ppt, ratio 1,700; HpCDD, Spanish ~ 40,000 ppt, reference ~36 ppt, ratio 1,000; HxCDDs, Spanish ~2,000 ppt, reference ~33 ppt, ratio 60.

3.2.2. *T-helper lymphocytes (CD4⁺CD45R0⁺) and activation markers (CD29, CD11a)*

Among the 4 adults with body burdens of HpCDD between 14,000 and 66,000 pg/g lipid and of OCDD between 380,000 and 660,000 pg/g lipid, no volunteer showed a reduced number of lymphocytes expressing the CD4CD45R0-receptors, as well as a reduced number of CD4⁺CD45R0⁺CD29^{hi} or the CD4⁺CD29⁺ cells (Table 6a), that were the most obvious indications of TCDD exposure observed in the monkeys. This lack of similarity with monkey data agrees with the results of residents with high exposures in the Seveso area.

Within the group of younger family members (n = 9), the lymphocytes of a 16 year-old boy (M_L) exhibited values for the percentage of cells expressing the CD45R0

the CD45R0CD29 as well as the CD29 receptors which were just below the reference range (Table 6b). Although being in the direction to be expected from the studies in monkeys this should be interpreted cautiously since the few volunteers were evaluated and the body burdens were low. Furthermore, the absolute number of these cells was normal, partly compensated for by an increased total lymphocyte count. Because the percentages of the memory-type helper cells of the younger volunteers were located within the lower half of the reference range, the mean value for this group (n = 9) was calculated to be significantly lower (19.4%) than that of the matched controls (24.1%) (Table 7). However, multi-regression analysis including the highly exposed persons did not recognize PCDDs as a contributing factor. In two of the younger volunteers (M_H and F_M) an increase in the $CD45R0^+CD29^+$ cells was measured, but none of these receptors alone, as well as the percentages, were outside the reference range (Table 6b). In all of the participants, there was no effect on the expression of the CD4CD11a receptors (Table 6b).

3.2.3. *T-helper lymphocytes ($CD4^+CD45RA^+$), the naive-type cells*

In previous studies, a decrease in the number of $CD4^+CD45RA^+$ lymphocytes (opposite to the clear-cut effect seen in monkeys) was observed in TCDD-exposed persons (Neubert et al., 1999a; Neubert et al., 1999b; Neubert et al., 2002). Within the subgroup of 4 older volunteers, one man and one woman showed a reduced number of $CD4^+CD45RA^+$ lymphocytes (Table 6a). In both cases the difference was borderline and it concerned only the absolute (M_E) or the percentage value (F_A). It is important to note that the reference range was defined as the 10-90% level of apparently healthy persons, and 20% of the data of a examined group may lie outside of this range. A decrease in the number of $CD4^+CD45RA^+$ lymphocytes would be opposite to the effect expected from the data in monkeys, but would be in accord with volunteers highly exposed to TCDD (>3,000 pg/g lipid). The number of our volunteers was too small for far reaching conclusions on this subtle change.

There was no indication of changes in lymphocytes with the CD45RA-receptors on $CD4^+$ cells in the group of younger volunteers (Table 6b). Except for one borderline effect (F_I), all values were within the reference range. Calculating a ratio may provide a sensitive measure of a trend. All values of the sensitive ratio: $CD45R0^+/CD45RA^+$ stayed well within the reference range of 0.2-2.2 for the members of the younger

group. However, of the 4 older persons this ratio was 4.5 (M_E) and 5.3 (F_A) for the two volunteers with reduced $CD45R0^+$ -values.

In summary, there was no indication of a change in the memory-type lymphocytes, assumed to be the cells specifically primed with an antigen. In 2 of the 4 older persons with the high exposure, the $CD4^+CD45RA^+$ lymphocyte subpopulation was minimally reduced, and the ratio $CD45R0^+/CD45RA^+$ was clearly outside the reference range. This may point to a trend similar to that observed previously at the high exposure to TCDD (>3,000 ppt in lipid) (Neubert et al., 1999a; Neubert et al., 1999b; Neubert et al., 2002). Especially low values of the transitional memory-type cells ($CD4^+CD45R0^+CD45RA^+$), which are thought to represent a stage in the transformation of naive to memory cells, were seen for 7 of the 9 members of the younger group (Table 6b). However, these cells are already rare under normal conditions (0.5-8% of lymphocytes) and it seems unlikely that this finding represented immunosuppression or an adverse health effect, rather than reflecting a regional or lifestyle difference from the reference group.

3.2.4. B cells ($CD20^+$) and the T/B ratio

We found no deviation of the percentage or the absolute number of B cells in peripheral blood of the exposed persons although this is a response to TCDD in the monkey (Tables 6a and 6b). The ratio T/B cells ($CD3^+/CD20^+$) of all exposed persons stayed within the large reference range except for one highly exposed volunteer (F_A) with a borderline value.

3.2.5. Cytotoxic and suppressor cells ($CD8^+CD56^+/57^+$, $CD8^+CD56^-/57^-$)

When evaluating the two subpopulations of the $CD8^+$ cells (Table 7), there appeared to be a trend opposite to that expected from studies in monkeys i.e. a decreased number of cytotoxic cells ($CD56^+CD57^+$), and an increased number of suppressor cells ($CD56^-CD57^-$). However, the ratio: $CD56^+/CD56^-CD57^-$ stayed within the large span of the reference range. Multi-regression analysis did not recognize dioxins as a contributing factor (Table 8). With respect to the ratio $CD4^+/CD8^+$, we observed high values (outside the reference range of 0.6-2.3) in two volunteers with the high body burdens (M_D and F_N), with ratios of 3.1 and 2.4, respectively. There was no alteration in the number or percentage of killer cells ($CD3^-CD56^+CD16^+$ or $CD3^-CD56^+CD16^-$) in the blood of the exposed family members.

3.2.6. Total lymphocytes

The average number of total lymphocytes in younger volunteers was found to be increased, the lymphocyte count of 4 out of the 9 participants being outside the reference range, despite the fact that these persons exhibited the lowest PCDD body burdens (Tables 6b and 7). The increased total number of lymphocytes apparently also influenced the absolute number of total T-lymphocytes (CD3⁺) and suppressor/cytotoxic cells (CD8⁺). There was less influence on the percentage of subpopulations. There was no indication of an infection. The lymphocyte count of the parents with the high body burdens were within the reference range. In a Seveso study, people exposed as children to TCDD also showed increased values of total lymphocytes for some years following the exposure (Mocarelli et al., 1992). However, these children all exhibited high TCDD body burdens.

3.2.7. Confounding factors in multi-regression analysis

One aim of our studies was to find whether changes in components and functions of the immune system were considered as reproducible and early markers of an exposure to highly chlorinated PCDDs and PCDFs. The immune system responds to a variety of stimuli and offers various defence strategies such that all deviations from a reference range cannot be considered as pathological. Observations in humans are difficult or impossible to be interpreted if important confounding factors, such as age, inter- and intra-individual variability, smoking and infections are not taken into account and the situation with PCDDs and PCDFs is further complicated by the exposure to mixtures of the congeners. These are especially difficult to control for when the number of exposed subjects is small. Analyses of variance indicated statistically significant differences between some lymphocyte subpopulations and the age-matched reference group (Table 7). Since all our data were evaluated both as absolute values (cells/ μ L blood) and as % of the total lymphocytes, the significance in some cases concerned only one of these evaluations. This was considered of minor relevance, especially when the number of cells per μ L blood was normal. Multi-regression analyses often confirmed an influence of age (Table 8) but for none of the lymphocyte subpopulations were dioxins shown clearly to be an influencing factor.

3.2.8. Correlation of analytical with medical data

The use of TEFs for estimating body burdens would greatly facilitate an association of medical endpoints with internal exposure. Unfortunately, this is not presently possible (see paragraph: 3.1.4). Concentrations of the congeners at the site of the lymphocyte surface receptors (or even within the thymus), or of the receptor-complex at the lymphocyte genome remain unknown. If one assumes the presently suggested TEFs-WHO for assessing body burdens, the TEQ within the adult members of Family 2 would amount to about 1,000 pg/g lipid (Table 2) at the time of the immunological investigations (1996). With the present degree of uncertainty, we suggest that almost all of this TEQ consists of PCDDs of hexa- or higher chlorinated congeners with little contribution from tetra- and penta-chlorinated PCDDs (< 4%).

Nonspecific deviations from the reference range for immune components have been reported in highly exposed volunteers at levels of about 3,000 pg TCDD/g lipid, and above (Neubert et al., 1999a; Neubert et al., 1999b; Neubert et al., 2002). In a study on workers exposed to PCDD/PCDFs plus PCBs, the same trend was observed in volunteers with TCDD body burdens of 760 ppt or less, in blood lipids (A. Kocan, C. Rappe and D. Neubert, paper in preparation). We conclude that the body burdens of the higher chlorinated congeners in the Spanish families are not much more potent than levels of 3,000 ppt TCDD in this respect. A transient earlier effect at a period with still higher body burdens cannot be excluded in our volunteers. Noteworthy is the increase in total lymphocytes in some of the volunteers exposed as children to the pollutants (Table 6b). However, this result predominantly concerns the family members with the lowest body burdens. Although in the same direction of some of the children exposed at Seveso, Italy, we have at present no explanation for this odd finding although it may reflect critical age at exposure. The two younger members of Family 2, with the highest body burdens, show normal values but were exposed at older ages than those of Family 1.

3.2.9. Proliferative capacity of lymphocytes in-vitro

Results of the lymphocyte proliferative responses after stimulation with various stimuli (see Methodology) were difficult to interpret due to the small number of volunteers studied, the considerable variability of the proliferative response and the confounding factors. With caution it can be concluded that the increased body burdens of the participants in the study did not induce clear-cut alterations in the capacity of the blood lymphocytes for proliferation (data not shown). Unfortunately,

a prolonged response to tetanus vaccination observed in persons highly exposed to TCDD (Mocarelli et al., 1992) could not be evaluated here since no data on the immunization status were available.

4. Conclusions

The data suggest (Rappe et al., 1998; Rodriguez-Pichardo et al., 1991) that higher chlorinated congeners of PCDDs and PCDFs are capable of inducing chloracne. Body burdens predominantly consisted of hepta- and octachlorinated congeners, for which little information is available, and levels of the dioxins were very high (>500,000 ppt OCDD in some cases) although the early measurements closer to the time of exposure, when the skin lesions existed, also showed moderately increased levels of penta- and hexachlorinated congeners in the plasma. No family member exhibited marked body burdens of TCDD (Figure 1C and D). Family 1 also show increased body burdens of two children who were exposed from their mother either *in utero* or by breast feeding (i.e. 2 grandchildren). After exposure, resolution of the chloracne occurred within a few years at the most although the body burdens of the highly chlorinated PCDDs persisted. Despite the chloracne, we have no evidence that other detrimental health effects could be ascribed to the high levels of PCDDs and PCDFs. The rate of decline of the highly chlorinated congeners (HpCDD and OCDD) was much faster in the participants exposed as children, compared with the adults. This extends and is more complicated than similar data on TCDD body burdens in children. Thus in this respect, children are not necessarily more sensitive to exposure to PCDDs or PCDFs than adults and is in agreement with recently published estimations on Seveso children and German infants (Kerger et al., 2006; Leung et al., 2006). OCDD/ HpCDD or OCDD/ HxCDD ratios in contaminated oil and the resulting body burdens were orders of magnitude different, even soon after the exposure, and further changed with time reflecting congeners with different elimination half-lives.

Analysis of important components of the immune system, including lymphocyte surface markers for subsets of helper-cells, suppressor and cytotoxic cells and B cells showed no changes that were markedly outside of a reference range despite the continued high body burdens of higher chlorinated PCDDs. However, 4 out of 7 daughters and sons of Family 1 exhibited significantly increased total numbers of lymphocytes. The initial body burdens, right after the exposure are unknown within

the younger group. None of these small changes observed were deemed of clinical relevance. The two members of Family 2 exposed as young adults did not show such an effect, despite much higher actual body burdens at the time of analysis but not perhaps at exposure. Although our investigations were performed long after the chloracne had resolved, other possible contaminants (e.g. pentachlorophenol) were eliminated, but the body burdens of highly chlorinated PCDDs persisted.

We conclude that extremely high body burdens in HpCDD (220-37,000 pg/ g lipid) and OCDD (3,000-660,000 pg/ g lipid) at the time of our study did not induce pronounced pathological alterations in those immunological components and functions that were measured. Transient effects on the immune system at the initial period with even higher body burdens cannot be excluded, but must have been reversible. For comparison, the background body burden in the German Federal Republic at about the same time was reported as 40 pg HpCDD/g lipid and 250 pg OCDD/g blood lipids (Beck et al., 1991; Pöpke et al., 1994). With the higher PCDDs we did not find more pronounced effects on the immunological components than in persons reported with body burdens of 3,000 pg TCDD/ or 600-750 pg TCDD/g blood lipids. In 2 of the 4 Spanish parents, who exhibited especially high body burdens, the CD4⁺CD45RA⁺ lymphocyte subpopulation (naive-type cells) was minimally reduced, but the ratio CD45RO⁺/CD45RA⁺ was outside the reference range, a finding similar to a trend seen in volunteers with high TCDD exposure (Neubert et al., 1999a; Neubert et al., 1999b; Neubert et al., 2002;) and workers exposed to PCDD/PCDFs plus PCBs (A. Kocan et al., paper in preparation). With some assumptions, the body burdens of the parents in our study would seem to be in the same range or even greater (Table 5) than body burdens that in monkeys induced clear-cut changes in the immunological variables investigated. Our data on the immunological components are consistent with low TEFs for the hepta- and octachlorinated PCDDs in humans, with the caveat that ratios of congeners to each other, as well as resulting TEQs, may differ between tissues.

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Figure legends

Figure 1. Analysis of polyhalogenated chemicals in olive oil apparently responsible for poisoning of Family 1 and their levels in a pooled blood sample from four members taken five years later (Rodriguez-Pichardo et al., 1991). A. PCDDs in oil. B. PCDFs in oil. C. PCDDs in pooled sera. D. PCDFs in pooled sera. OCDD relative to HpCDDs measured in the oil was much greater than observed for serum lipids. One explanation might be the high insolubility of OCDD influencing both sampling of the oil and absorption from the intestine by the family members.

Figure 2. Analyses of polyhalogenated chemicals in olive oil sample apparently responsible for the poisoning of Family 2. A. PCDDs. B. PCDFs.

Table 1. Comparison of blood PCDDs in members of Family 1 in 1990 and in 1996.

Sex	M _E ^a	F _N	M _F ^a	F _G ^a	F _J ^a	M _H	M _K	F _M	M _L	F _x ^b	F _y ^b
1990											
Age	50	47	20	19	17	16	12	10	8		
Wt (kg)	100	80	54	54	56	51	43	35	22		
PCDDs (pg/g lipid)											
2378-TCDD	2.8	ND	ND	ND	ND	ND	ND	ND	ND		
12378-PeCDD	77	28	71	25	24	22	ND	ND	ND		
123478-HxCDD	220	77	240	78	71	52	30	32	29		
123678-HxCDD	860	360	1100	370	350	370	180	180	50		
123789-HxCDD	920	330	230	140	200	50	48	26	ND		
1234678-HpCDD	46000	16000	14000	8200	9400	2100	2600	1800	1600		
OCDD	300000	240000	210000	180000	140000	20000	28000	17000	14000		
<i>TEQ (WHO 2006)</i>	<i>830</i>	<i>337</i>	<i>431</i>	<i>220</i>	<i>222</i>	<i>96</i>	<i>60</i>	<i>50</i>	<i>28</i>		
1996											
Age	56	53	26	25	23	22	18	16	13	3	2
Wt (kg)	102	70	60	63	58	65	68	53	43	-	-
PCDDs (pg/g lipid)											
2378-TCDD	2	<1	<1	<1	<1	<1	<1	<1	<1	<9.9	<8.0
12378-PeCDD	54	29	16	9	21	9	8	8	6	45	30
123478-Hx	150	76	53	32	59	12	18	16	<6	110	27
123678-HxCDD	640	400	240	150	310	150	130	100	45	350	140
123789-HxCDD	880	400	32	68	210	20	30	30	28	160	42
1234678-HpCDD	37000	14000	1400	3100	4600	190	390	420	220	2600	720
OCDD	660000	380000	37000	110000	130000	6100	13000	9000	3000	45000	11000
<i>TEQ (WHO 2006)^c</i>	<i>791</i>	<i>371</i>	<i>73</i>	<i>98</i>	<i>164</i>	<i>31</i>	<i>34</i>	<i>30</i>	<i>16</i>	<i>151</i>	<i>66</i>

^a Family members that provided blood for pooled sample in 1987. ^b Children of eldest daughter. Only 5ml of blood was taken so that precision of low contaminants was weakened. ND. ^c TEQ gives the total "Toxic equivalents to TCDD", using WHO TEFs (van den Berg et al., 2006). Note: TEQ-values are not verified for human body burdens. ND, Not detected. M and F are male and female respectively. Subscripts are individual codes.

Table 2. Comparison of the PCDDs in Family 2 in 1990 and in 1996.

	1990					1996				
	M _D	F _A	M _Z	F _B	M _C	M _D	F _A	M _Z ^a	F _B	M _C
Sex										
Age	58	55	31	27	17	64	61	37	33	23
Wt (kg)	74	75	67	58	83	72	73	-	56	90
PCDDs and PCDFs (pg/g lipid)										
2378-TCDD	1.7	ND	ND	ND	ND	3.4	<1	-	<1	<1
12378-PeCDD	32	12	13	28	11	34	13	-	14	7.2
123478-HxCDD	150	52	55	94	17	140	61	-	49	22
123678-HxCDD	3500	1700	1300	2700	1700	2700	1500	-	1400	1000
123789-HxCDD	1200	480	360	800	390	810	550	-	250	150
1234678-HpCDD	54000	62000	53000	84000	64000	58000	66000	-	26000	16000
OCDD	170000	190000	190000	280000	180000	490000	440000	-	330000	180000
2378-TCDF	2.6	4.9	1.7	3.6	3.1	2.9	4.1	-	3.2	7.5
23478-PeCDF	26	16	6.1	18	25	30	21	-	14	25
123478-HxCDF	44	27	18	36	23	29	21	-	14	10
123678-HxCDF	17	11	6.5	13	8.8	13	9.0	-	6.00	5.0
234678-HxCDF	50	37	20	30	22	11	8.8	-	5.6	5.1
1234678-HpCDF	2300	2400	2100	4000	3000	1600	1600	-	1000	840
1234789-HpCDF	27	26	13	32	21	9	7	-	3	2
OCDF	1200	1800	1200	1700	1300	NA	NA	-	NA	NA
TEQ (WHO 2006) ^b	1148	950	787	1311	968	1160	1044	-	559	356

ND: Not detected. NA: Not analysed as levels were too low. ^a The eldest son was unable to attend the clinic for blood collection.

TEQ gives the total "Toxic equivalents to TCDD", using WHO TEFs (van den Berg et al., 2006). Note: TEQ-values are not verified for human body burdens. Other PCDFs in the 1996 samples were detected but were of negligible significance. M and F are male and female respectively. Subscripts are codes of individual.

Table 3. Ratio of various PCDDs within the body burden of the volunteers.

Family member	Ratio		Ratio		Ratio		Ratio		Ratio	
	OC/ HpC		OC/ Hx6C		HpC / Hx6C		Hx6C / Hx4C		OC/ PeC*	
	1990	1996	1990	1996	1990	1996	1990	1996	1990	1996
Family 1										
M _E ¹	6.5	17.8	349	1031	53.5	57.8	3.9	4.3	3900	12000
F _N ¹	15.0	27.1	667	950	44.4	35.0	4.7	5.3	8600	13000
M _F ²	15.0	26.4	191	154	12.7	5.8	4.6	4.5	2900	1300
F _G ²	22.0	35.5	487	733	22.2	20.7	4.7	4.7	7200	12000
F _J ²	14.9	28.3	400	419	26.9	14.8	4.9	5.3	5800	6200
M _H ²	9.5	32.1	54	41	5.7	1.3	7.1	12.5		680
M _K ²	10.8	33.3	156	100	14.4	3.0	6.0	7.2		1600
F _M ²	9.4	21.4	94	90	10.0	4.2	5.6	6.3		1100
M _L ²	8.8	13.6	280	67	32.0	4.9	1.7	>7.5?		500
F _x ^{3 4}		17.3		129		7.4		3.2		1000
F _y ^{3 4}		15.3		79		5.1		5.2		367
(mean ± SD): 12.4 ± 4.7							4.8 ± 1.5			
(mean ± SD): 26.2 ± 7.3							6.3 ± 2.7			
Family 2										
M _D ¹	3.2	8.5	48.6	181.5	15.4	21.5	23.3	19.3	5300	14000
F _A ¹	3.1	6.7	111.8	293.3	36.5	44.0	32.7	24.6	16000	34000
F _B ²	3.3	12.7	103.7	235.7	31.1	18.6	28.7	28.6	10000	23500
M _C ²	2.8	11.3	105.9	180.0	37.6	16.0	10.0	45.5	16000	25000
M _Z ⁴	3.6		146.2		40.8		23.6		15000	

Persons with exposure to the same source (Family 1 *versus* Family 2) exhibit surprisingly similar ratios, which vary from 1990 to 1996 by no more than a factor of < 2-3. The ratio between two hexa-chlorinated congeners (Hx6C / Hx4C) shows the least variability. The ratio within the human blood lipid differs greatly from that within the corresponding oil sample.

* values were rounded up. ¹ parents, ² children, ³ grandchildren (exposure *in utero* and breast-fed), ⁴ no immunology. OC: OCDD, HpC: HpCDD, Hx4C: 1,2,3,4,7,8 HxCDD, Hx6C: 1,2,3,6,7,8 HxCDD, PeC: 1,2,3,7,8, PeCDD,

Table 4. Comparison of the general (German) population with an example of the high values of a member of the two Spanish families exposed accidentally to PCDDs.

		General population (1995)* pg/g	Highest values of Spanish families				
			absolute (1996) pg/g	x WHO TEFs, 2006 abs.	%	x NAT/CCMS I-TEFs, 1988 abs.	%
2378-	TCDD	2.7	3.4	3.4	0.3 %	3.4	0.2 %
12378-	PeCDD	5.6	34	34	3.0 %	17	1.2 %
Sum	HxCDDs	32.9	3,650	365	31.9 %	365	25.1 %
1234678-	HpCDD	36.3	58,000	580	50.7 %	580	39.9 %
12346789-	OCDD	331	490,000	163	14.2 %	490	33.7 %
	<i>SUM PCDDs**</i>	<i>420.3</i>	<i>ca 550,000</i>	<i>1,1451</i>	<i>100 %</i>	<i>1,455</i>	<i>100 %</i>
-	<i>SUM PCCFs</i>	<i>42.6</i>			**		**
-	<i>SUM PCCDs/PCDFs</i>	<i>480.0</i>					

The exposed person exhibited an about 2000-fold higher body burden of OCDD when compared with the median value of the general German population. Results of multiplying body burdens with present TEFs do not have a true scientific justification, and results of calculations critically depend on the selection of different TEFs.

* Median values, 134 (86) blood samples of the general *German* population (values in pg/g lipid [ppt] from (Päpke et al., 1994; Päpke et al., 1995)).

** If TEFs (for PCDDs) are applied for the data of the general population, values of 12.1 (WHO 2006-TEFs (van den Berg et al., 2006)) or 9.5 (NATO/CCMS, 1988 I-TEFs (NATO/CCMS, 1988)) result as a "background".

PCDFs have not been measured regularly, but they seem to be of minor significance.

Table 5. Attempt to compare human data with those obtained in *Callithrix*.

Congener	Species	Single dose of	Measured or assumed body burden (adipose tissue)	Effect on ¹ CD4 ⁺ CD45R0 ⁺
<i>Measurements</i>				
2378- TCDD	<i>Callithrix</i> ²	10 ng/kg bw	80 ppt	LOEC ²
2378- TCDD	<i>Callithrix</i> ³	30 ng/kg bw	250 ppt	pronounced effect
2378- TCDD	Human ⁴	10 ng/kg bw	30 ppt	
2378- TCDD	Human ⁴	27 ng/kg bw	80 ppt	
2378- TCDD	Human ⁵		250 ppt	no typical effect
2378- TCDD	Human ⁶		3,000 ppt	some trend in males ?
2378- TCDD	Human ⁷		≤ 760 ppt	some trend in workers
<i>Assumptions</i>				
OCDD	Human ⁸	TEF (WHO 2006) 80 x 3333	270,000 ppt	???
OCDD	Human ⁹	TEF (WHO 2006) 250 x 3333	830,000 ppt	???
OCDD	Human ¹⁰	TEF (NATO/CCMS 1988) 250 x 1000	250,000 ppt	???
<i>Measurements</i>				
OCDD	Human ¹¹	This study, body burden up to	500,000 ppt	a small trend ?
TEQ	Human ¹²	This study, (van den Berg et al., 2006)	1,000 ppt	a small trend ?

¹ Surface receptor on lymphocytes (helper-inducer, memory cells), down-regulated in *Callithrix*.

² Body burden achieved at this dose. Lowest dose with clear-cut effect of TCDD.

² LOEC: lowest observed effect concentration (body burden in adipose tissue).

³ Body burden with very pronounced effect on these lymphocyte subpopulations.

⁴ Body burden in humans after 10 mg TCDD/kg bw (from data on 1 ng/kg bw [(Poiger and Schlatter, 1986)]).

⁵ Calculation from 1 ng/kg bw (Poiger and Schlatter, 1986) to LOEC in monkeys.

⁶ Trend for decrease in CD4⁺CD45RA⁺ (opposite of expected) in *males* only above this body burden, but almost all values stay within the reference range (Neubert et al., 1999a; Neubert et al., 1999b).

⁷ Trend for decrease in CD4⁺CD45RA⁺ increase in CD4⁺CD45R0⁺ (opposite of expected) in *workers*, but almost all values stay within the reference range; poly-exposure {A. Kocan et al., unpublished data}.

⁸ Body burden for OCDD assuming the TEF of 0.0003 {van den Berg, 2006 #18} x data (monkeys) of # 2.

⁹ Body burden for OCDD assuming the TEF of 0.0003 (van den Berg et al., 2006) x data (monkeys) of # 3.

¹⁰ Body burden for OCDD assuming the TEF of 0.001 (NATO/CCMS, 1988) x data (monkeys) of # 3.

¹¹ Body burden for OCDD reached in the adults of the two Spanish families (Tables 1 and 2) (compare with # 7-9).

¹² Body burden extrapolated with TEFs WHO-2006, adults of the two Spanish families (compare with # 3).

Table 6a. Analysis of lymphocyte subsets from the older volunteers exposed to highly chlorinated PCDDs from contaminated cooking oil.

Lymphocyte subpopulations ¹	Age→	Older Volunteers				References ²	
		F _A 61	M _D 64	M _E 56	F _N 53	Min	Max
Leukocytes	<i>n</i>	6400	7600	6200	7000	4800	10000
Lymphocytes	<i>n</i>	2400	2196	2003	2702	1400	4000
Lymphocytes	% <i>leuco</i>	37.5	28.9	32.3	38.6	25	45
CD3 ⁺ ³	%L	73.1	78.7	63.0	77.1	59.9	91.3
	<i>n</i>	1754	1727	1263	2084	831	2686
CD4 ⁺ ⁴	%L	35.4	56.7	37.1	55.4	25.3	60.6
	<i>n</i>	850	1245	743	1497	443	1766
CD4 ⁺ CD45R0 ⁺ ⁵	%L	29.3	30.0	28.3	26.5	13.9	37.8
	<i>n</i>	704	658	567	715	262	940
CD4 ⁺ CD45R0 ⁺ CD29 ^{hi}	%L	21.8	22.2	23.1	20.8	8.2	29.3
	<i>n</i>	523	488	462	561	204	772
CD4 ⁺ CD29 ^{hi} ⁶	%L	26.3	28.0	25.4	25.7	16.6	39.7
	<i>n</i>	632	615	509	695	282	1119
CD4 ⁺ CD45RA ⁺ ⁷	%L	5.0	22.6	6.3	26.6	5.7	48.8
	<i>n</i>	120	496	126	666	131	1024
CD4 ⁺ CD45R0 ⁺ CD45RA ⁺ ⁸	%L	0.7	0.6	0.1	2.3	0.5	7.5
	<i>n</i>	16	13	1	61	9	141
CD8 ⁺ ⁹	%L	40.7	18.1	29.0	22.7	17.4	45.0
	<i>n</i>	977	397	581	613	209	1425
CD8 ⁺ 45R0 ⁺ 44 ⁺ ¹⁰	%L	13.6	6.4	9.6	3.7	2.2	25.7
	<i>n</i>	325	141	191	100	42	782
CD8 ⁺ 56 ⁺ 57 ⁺ ¹¹	%L	9.7	1.9	6.3	7.2	0.7	16.6
	<i>n</i>	232	42	126	195	8	446
CD8 ⁺ 57 ⁺	%L	17.6	4.7	13.5	11.9	5.5	26.8
	<i>n</i>	422	102	270	321	66	615
CD8 ⁺ 56 ⁺ 57 ⁻ ¹²	%L	20.3	10.1	12.4	8.5	8.1	29.7
	<i>n</i>	488	222	248	231	133	819
CD20 ⁺ ¹³	%L	21.4	15.1	9.2	4.5	2.7	33.9
	<i>n</i>	513	331	184	122	57	671
CD3 ⁻ CD56 ⁺ CD16 ⁻ ¹⁴	%L	4.1	0.8	0.9	1.1	0	8.7
	<i>n</i>	98	18	19	30	1	278
CD3 ⁻ CD56 ⁻ CD16 ⁺ ¹⁴	%L	0.5	0.7	0.3	0.3	0	3.4
	<i>n</i>	12	16	6	7	0	108

¹ all % values of lymphocyte subsets: % of *total* lymphocytes, all *n*-values : cells / μ L blood. ² reference range: age-matched from a northern Italian reference population (n = 255), 5-95%. ³ total T-cells; ⁴ total helper cells; ⁵⁻⁸ different helper cell subsets; ⁵ memory-type cells, ⁶ cells with β 1-integrin common β -chain, activation marker; ⁷ naive-type cells, ⁸ transitional cells, from naive to memory cells; ⁹⁻
¹² different suppressor/cytotoxic subsets; ¹⁰ memory-type CD8-cells, ¹¹ cytotoxic cells with two typical

markers; ¹² suppressor cells with two typical markers; ¹³ B cells; ¹⁴ killer-cells. Values in bold are outside of the reference range.

Table 6b. Analysis of lymphocyte subsets from the younger volunteers exposed to highly chlorinated PCDDs from contaminated cooking oil.

Lymphocyte subpopulations ¹	Age→	Younger Volunteers									References ²	
		F _B	M _C	M _F	F _G	M _H	F _J	M _K	M _L	F _M	Min	Max
		33	23	26	25	22	23	18	16	13		
Leukocytes	<i>n</i>	5100	7600	8100	7800	13600	7600	10100	7200	11600	4800	10000
Lymphocytes	<i>n</i>	2300	1497	2600	2902	5494	2903	4000	3701	4396	986	2774
Lymphocytes	% <i>leuko</i>	45.1	19.7	32.1	37.2	40.4	38.2	39.6	51.4	37.9	20	45
CD3 ⁺ ³	% <i>L</i>	81.2	73.8	80.0	75.6	82.7	85.6	70.2	77.8	80.0	69.6	90.2
	<i>n</i>	1868	1104	2080	2192	4544	2485	2810	2878	3517	986	2774
CD4 ⁺ ⁴	% <i>L</i>	49.6	48.5	35.3	40.9	44.2	34.7	41.4	36.8	44.3	29.1	57.3
	<i>n</i>	1141	726	918	1187	2428	1007	1656	1362	1947	494	1775
CD4 ⁺ 45R0 ⁺ ⁵	% <i>L</i>	24.7	24.3	15.9	18.5	19.8	22.0	15.2	9.8	21.5	13.0	37.4
	<i>n</i>	569	363	414	537	1088	639	609	363	947	239	1157
CD4 ⁺ 45R0 ⁺ 29 ⁺ hi	% <i>L</i>	17.7	21.8	11.0	13.7	15.6	18.7	12.7	8.8	19.4	9.9	27.2
	<i>n</i>	408	327	287	398	857	544	508	326	853	185	745
CD4 ⁺ CD29 ⁺ hi ⁶	% <i>L</i>	21.8	25.2	15.5	20.1	20.9	21.6	18.5	12.3	22.9	14.8	35.6
	<i>n</i>	500	377	403	584	1148	626	740	456	1008	251	1140
CD4 ⁺ 45R0 ⁺ 11a ⁺ hi ⁷	% <i>L</i>	19.1	19.9	11.0	15.2	16.8	16.9	12.3	6.9	18.4	6.3	29.7
	<i>n</i>	439	298	285	442	921	491	491	256	808	101	951
CD4 ⁺ 45RA ⁺ ⁸	% <i>L</i>	22.2	22.6	14.5	19.4	19.5	10.1	21.6	24.8	19.4	10.6	37.8
	<i>n</i>	511	339	377	563	1073	293	864	918	854	241	1144
CD4 ⁺ 45R0 ⁺ RA ⁺ ⁹	% <i>L</i>	1.2	0.5	0	0.3	0.1	0.3	0.2	0.5	0	0.5	8.4
	<i>n</i>	27	7	0	10	3	10	6	19	0	12	213
CD8 ⁺ ¹⁰	% <i>L</i>	32.3	29.2	33.4	33.0	39.6	44.4	28.7	37.1	34.7	24.2	54.7
	<i>n</i>	743	437	868	958	2176	1289	1148	1373	1525	370	1424
CD8 ⁺ 45R0 ⁺ 44 ⁺ ¹¹	% <i>L</i>	3.9	2.8	6.4	6.3	7.1	9.7	3.0	3.3	5.7	2.2	31.9
	<i>n</i>	90	42	166	184	389	282	120	122	249	60	1053
CD8 ⁺ 56 ⁺ 57 ⁺ ¹²	% <i>L</i>	1.2	2.0	7.1	6.0	4.1	13.3	1.8	3.5	2.7	2.6	27.2
	<i>n</i>	28	29	185	173	227	387	72	131	120	46	599
CD8 ⁺ 57 ⁺	% <i>L</i>	2.2	4.2	17.4	10.3	19.1	19.1	3.2	13.8	6.9	3.8	37.9
	<i>n</i>	51	63	453	300	1049	554	129	510	303	90	833
CD8 ⁺ 56 ⁺ 57 ⁺ ¹³	% <i>L</i>	27.2	22.0	13.8	19.8	19.1	20.2	21.9	21.7	24.9	1.2	29.4
	<i>n</i>	626	329	359	574	1052	587	876	803	1095	37	969
CD20 ⁺ ¹⁴	% <i>L</i>	11.1	12.3	4.5	4.5	6.5	2.9	8.3	4.3	6.7	2.5	20.3
	<i>n</i>	256	184	118	130	357	85	331	158	296	57	671
CD3 ⁺ CD56 ⁺ CD16 ⁺ ¹⁵	% <i>L</i>	1.2	1.8	1.8	2.3	1.7	1.2	0.6	0.4	0.7	0.1	12.0
	<i>n</i>	28	27	47	67	92	34	26	15	31	2	291
CD3 ⁺ CD56 ⁺ CD16 ⁺ ¹⁵	% <i>L</i>	0.4	0.9	0.6	0.4	0.3	0.1	0.4	0.6	0.2	0	2.5
	<i>n</i>	10	13	17	12	15	3	14	24	9	0	42

¹ all % values of lymphocyte subsets: % of *total* lymphocytes, all *n*-values: cells / μ L blood. ² reference range: age-matched from a northern Italian reference population (n = 255), 5-95%. ³ total T-cells; ⁴ total helper cells; ⁵⁻⁹ different helper cell subsets; ⁵ memory-type cells, ⁶ cells with β 1-integrin common β -

chain, activation marker; ⁷ cells with $\beta 2$ -integrin α -chain, activation marker; ⁸ naive-type cells, ⁹ transitional cells, from naïve to memory cells; ¹⁰⁻¹³ different suppressor cytotoxic subsets; ¹¹ memory-type CD8-cells, ¹² cytotoxic cells with two typical markers; ¹³ suppressor cells with two typical markers; ¹⁴ B cells; ¹⁵ killer-cells. Values in bold are outside of the reference range.

Table 6c. Analysis of lymphocyte subsets from Spanish volunteers with no special exposure to PCDDs. Four volunteers were recruited as references from the medical personal.

Lymphocyte Subpopulations ¹	Age→	Spanish Reference Volunteers				References ²	
		F _O 42	F _P 39	F _Q 31	F _R 49	Min	Max
Leukocytes	<i>n</i>	9800	8000	9700	6700	4800	10000
Lymphocytes	<i>n</i>	3097	3096	3298	2298	1400	4000
Lymphocytes	% <i>leuco</i>	31.6	38.7	34.0	34.3	25	45
CD3 ⁺ ³	%L	74.2	76.5	78.8	72.8	59.9	91.3
	<i>n</i>	2298	2368	2599	1673	831	2686
CD4 ⁺ ⁴	%L	45.9	50.8	45.3	55.7	25.3	60.6
	<i>n</i>	1422	1573	1494	1280	443	1766
CD4 ⁺ CD45R0 ⁺ ⁵	%L	17.1	25.3	25.1	24.6	13.9	37.8
	<i>n</i>	531	783	827	564	262	940
CD4 ⁺ CD45R0 ⁺ CD29 ⁺ hi	%L	13.7	19.7	18.9	19.4	8.2	29.3
	<i>n</i>	425	610	624	445	04	772
CD4 ⁺ CD29 ⁺ hi ⁶	%L	16.4	24.4	22.2	24.0	16.6	39.7
	<i>n</i>	508	754	731	552	282	1119
CD4 ⁺ CD45RA ⁺ ⁷	%L	26.3	19.4	14.8	27.1	5.7	48.8
	<i>n</i>	813	600	488	623	131	1024
CD4 ⁺ CD45R0 ⁺ CD45RA ⁺ ⁸	%L	0.6	0.9	0.5	1.4	0.5	7.5
	<i>n</i>	19	28	17	31	9	141
CD8 ⁺ ⁹	%L	27.7	26.1	33.8	17.2	17.4	45.0
	<i>n</i>	858	808	1115	395	209	1425
CD8 ⁺ 45R0 ⁺ 44 ⁺ ¹⁰	%L	1.6	7.9	5.8	5.1	2.2	25.7
	<i>n</i>	50	244	191	117	42	782
CD8 ⁺ 56 ⁺ 57 ⁺ ¹¹	%L	2.1	3.7	1.4	2.8	0.7	16.6
	<i>n</i>	66	114	46	65	8	446
CD8 ⁺ 57 ⁺	%L	5.8	6.3	12.1	3.8	5.5	26.8
	<i>n</i>	178	194	398	86	66	615
CD8 ⁺ 56 ⁻ 57 ⁻ ¹²	%L	20.1	16.4	20.1	10.8	8.1	29.7
	<i>n</i>	624	508	664	247	133	819
CD20 ⁺ ¹³	%L	16.9	5.9	6.2	9.0	2.7	33.9
	<i>n</i>	522	184	204	207	57	671
CD3 ⁻ CD56 ⁺ CD16 ⁻ ¹⁴	%L	0.9	2.3	0.5	2.1	0	8.7
	<i>n</i>	28	72	16	47	1	278
CD3 ⁻ CD56 ⁻ CD16 ⁺ ¹⁴	%L	0.4	0.7	2.1	0.4	0	3.4
	<i>n</i>	12	23	70	9	0	108

¹ all % values of lymphocyte subsets: % of *total* lymphocytes, all *n* -values : cells / μ L blood. ² reference range: age-matched from a northern Italian reference population (*n* = 255), 5-95%. ³ total T-cells; ⁴ total helper cells; ⁵⁻⁸ different helper cell subsets; ⁵ memory-type cells, ⁶ cells with β 1-integrin common β -chain, activation marker; ⁷ naive-type cells, ⁸ transitional cells, from naive to memory cells; ⁹⁻¹² different suppressor/cytotoxic subsets; ¹⁰ memory-type CD8-cells, ¹¹ cytotoxic cells with two typical

markers; ¹² suppressor cells with two typical markers; ¹³ B cells; ¹⁴ killer-cells. Values in bold are outside of the reference range.

Table 7. Analysis of variance of the immunological data of the younger volunteers.

Lymphocyte Subpopulations	Effect		Reference persons mean $\pm$ SD (n = 44)	Exposed volunteers mean $\pm$ SD (n = 9)	Difference P =
Lymphocytes	$\uparrow$	n	2,382 $\pm$ 552	3,311 $\pm$ 1,211	0.001
CD3 ⁺	$\uparrow$	n	1,879 $\pm$ 464	2,609 $\pm$ 999	0.001
CD8 ⁺	$\uparrow$	n	821 $\pm$ 241	1,168 $\pm$ 507	0.002
CD4 ⁺ CD45R0 ⁺	$\downarrow$	%	24.1 $\pm$ 6.1	19.4 $\pm$ 4.9	0.035
CD4 ⁺ CD29 ⁺ hi	$\downarrow$	%	24.0 $\pm$ 4.5	19.9 $\pm$ 3.9	0.013
CD4 ⁺ CD11a ⁺ hi	$\uparrow$	n	440 $\pm$ 193	631 $\pm$ 283	0.017
CD8 ⁺ CD56 ⁺ CD57 ⁺	?	%	7.9 $\pm$ 5.0	4.6 $\pm$ 3.8	0.073
CD8 ⁺ CD56 ⁺	$\downarrow$	%	13.9 $\pm$ 7.1	7.5 $\pm$ 4.5	0.013
CD8 ⁺ CD56 ⁻ CD57 ⁻	$\uparrow$	%	16.3 $\pm$ 5.9	21.2 $\pm$ 3.8	0.020
CD8 ⁺ CD56 ⁻ CD57 ⁻	$\uparrow$	n	388 $\pm$ 176	700 $\pm$ 276	0.000
CD8 ⁺ CD45R0 ⁺ CD44 ⁺	$\downarrow$	%	12.9 $\pm$ 7.0	5.4 $\pm$ 2.3	0.003
CD8 ⁺ CD45R0 ⁺ CD44 ⁺	?	n	304 $\pm$ 184	182 $\pm$ 108	0.062

Absolute (n = cells/ μ L) and relative (% of total lymphocytes) were evaluated of the younger volunteers only. The 9 exposed volunteers were compared with 44 age-matched reference persons (from northern Italy). Compiled are only data showing differences. For explanation of the various lymphocyte subsets see legend to Table 6.

Table 8. Multi-regression analysis of the immunological data.

Surface receptor	Influence of known factors on regression curve					
	Regression curve			Age	Cigarettes	BL ¹ PCDDs
	n	%	r ² [adj.] P =	P =	P =	P =
Lymphocytes	n	57 %	0.00	Ø	↑ 0.01	Ø
CD3 ⁺ ²	%	3 %	0.35	Ø	Ø	Ø
CD3 ⁺	n	97 %	0.00	Ø	Ø	↑ 0.00
CD4 ⁺	%	7 %	0.27	Ø	Ø	Ø
CD4 ⁺	n	83 %	0.00	↑ 0.04	Ø	↑ 0.00
CD4 ⁺ CD45R0 ⁺	%	48 %	0.01	↑ 0.01	Ø	Ø
CD4 ⁺ CD45R0 ⁺	n	54 %	0.01	↑ 0.01	Ø	↑ 0.02
CD4 ⁺ CD29 ⁺ hi	%	32 %	0.04	↑ 0.03	Ø	Ø
CD4 ⁺ CD29 ⁺ hi	n	67 %	0.00	↑ 0.04	Ø	↑ 0.00
CD4 ⁺ CD45R0 ⁺ CD29 ⁺ hi	%	27 %	0.06	↑ 0.04	Ø	Ø
CD4 ⁺ CD45R0 ⁺ CD29 ⁺ hi	n	49 %	0.01	↑ 0.04	Ø	↑ 0.02
CD4 ⁺ CD45R0 ⁺ CD11a ⁺ hi	%	21 %	0.10	Ø	Ø	Ø
CD4 ⁺ CD45R0 ⁺ CD11a ⁺ hi	n	38 %	0.04	Ø	Ø	Ø
CD4 ⁺ CD45RA ⁺	%	00 %	0.55	Ø	Ø	Ø
CD4 ⁺ CD45RA ⁺	n	35 %	0.05	Ø	Ø	Ø
CD8 ⁺	%	35 %	0.03	↓ 0.01	Ø	Ø
CD8 ⁺	n	87 %	0.00	↓ 0.01	Ø	↑ 0.00
CD8 ⁺ CD56 ⁺ CD57 ⁺	%	00 %	0.72	Ø	Ø	Ø
CD8 ⁺ CD56 ⁺ CD57 ⁺	n	00 %	0.69	Ø	Ø	Ø
CD8 ⁺ CD56 ⁻ CD57 ⁻	%	26 %	0.07	↓ 0.03	Ø	Ø
CD8 ⁺ CD56 ⁻ CD57 ⁻	n	79 %	0.00	Ø	Ø	↑ 0.00
CD3 ⁻ CD56 ⁺ CD16 ⁻	%	00 %	0.68	Ø	Ø	Ø
CD3 ⁻ CD56 ⁺ CD16 ⁻	n	00 %	0.86	Ø	Ø	Ø
CD3 ⁻ CD56 ⁻ CD16 ⁺	%	1 %	0.39	Ø	Ø	Ø
CD3 ⁻ CD56 ⁻ CD16 ⁺	n	00 %	0.44	Ø	Ø	Ø
CD20 ⁺	%	28 %	0.06	Ø	Ø	Ø
CD20 ⁺	n	30 %	0.07	↑ 0.045	Ø	Ø

Values were expressed as percentage of whole lymphocytes (%) and as absolute values (n = cells/ μ L). ↑ ↓ upward or downward trend, Ø no effect. ¹ Blood lymphocytes. ² For explanation of the various lymphocyte subsets see legend to Table 6.

Figure 1

A

B

C

D

Figure 2

A

B