EFFECTS OF DIOXINS IN HUMANS AND CORRELATION WITH ANIMAL DATA

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Peculiarities on the Toxicity of Dioxins:

The biological actions and the toxicity of dioxins are characterized by a number of peculiarities which must be taken into account. Without considering these specific and rather unusual aspects, a meaningful evaluation of possible adverse health effects for humans is impossible. Some problems, which typically occur when assessing the toxicity of "environmental" substances in general, are exaggerated in the case of the dioxins. It is essential to discuss these peculiarities in order to understand why so many misunderstandings and false conclusions with respect to the toxicity of these substances have accumulated during the past decades. Some of the peculiarities include:

- There are pronounced species differences in the **kinetics** between rodents and humans.
- In animal studies, TCDD is a "**carcinogen**," an "**anti-carcinogen**," or "**no carcinogen**" at all, critically depending on the dose and experimental conditions. The significance of this finding in relation to humans remains obscure, and animal data are of little help in predicting carcinogenicity of TCDD in humans.
- Typical effects on the **immune system** of TCDD in experimental animals cannot be reproduced in humans at appropriate exposure levels. Some of the observed effects on the human immune system rather appear as "favorable."
- Dose-response in animal tests was shown to be **non-linear** for several end points, and dose-dependent opposite effects were reported in experimental studies.
- The most sensitive biological effect of dioxins in animal studies, **enzyme induction** of hepatic monooxygenases in rodents and nonhuman primates, can be induced in humans, but the extent of induction is only similar to that produced by smoking.
- There is no scientific basis to using **I-TE factors** for quantitatively predicting toxicity in various organs of humans. Epidemiological human data on other "dioxins" are scanty.
- In epidemiological studies on TCDD exposure, numerous **confounding factors** (e.g. age, smoking habits, exposure to other chemicals) must be considered. This has not been possible in the majority of studies, greatly limiting the predictive values of such studies.

Some problems connected with these peculiarities during human risk assessment shall be discussed, and some of the common pitfalls and misinterpretations are compiled.

General Problems with Evaluating Human Data:

Questions on the possible toxicological hazard for humans can best be answered from human data. In fact, a "**risk assessment**" in the proper sense of the term can, *per definitionem,* **only** be performed on the basis of human data, and implies establishing a quantitative relationship between the extent of exposure and a defined adverse health effect (dose-response relationship).

This does by no means suggest that "**preventive hazard minimization**", i.e. the **extrapolation** of results from animal studies to the situation that might possibly be assumed to exist in humans, is unimportant. However, most often one or even several worse-case assumptions are made. Both types of strategies attempting to recognize, and hopefully prevent, further toxicological health hazards are important, but they are aimed at quite different goals and rely on vastly different strategies.

Although risk assessment proper solely relies on human data, the evaluation is by no means always easy and straightforward. For many reasons, the situation is comparatively easy in the case of medicinal drugs, and a vast and "classical" literature exists. With respect to "environmental substances," the main problems include:

a) The size of the human data pool is (in fact fortunately) often insufficient for any reliable and far reaching conclusions.
b) Data on body burdens and exposure levels are often insufficient, or even lacking.
c) Poly-exposure to substances of quite different classes had often occurred, but conclusions with respect to a single component present in this mixture are asked for.
d) Many confounding factors should, but cannot, be recognized and taken into account.

When the important prerequisites mentioned above are not fulfilled, a proper risk assessment is impossible, and attempts to define a toxicological risk for humans must remain speculative.

Special Situation and Problems with Dioxins:

The toxicity of "dioxins" has long been considered a problem, research being greatly stimulated after the accident in Seveso in 1976. Is exposure to dioxins, at the relevant levels of exposure, really a serious health problem? Is TCDD, as often stated, one of the most dangerous substances for humans, even at very low doses? In order to answer these questions properly, additional answers to further crucial questions with respect to “dioxins” must be found:

1. Are there sufficient human data available to allow a risk assessment?
2. Are there sufficient data on human exposure levels and body burdens available?
3. Which are the most significant toxicological endpoints to be considered?
4. Can the problem of poly-exposure be solved in human studies?
5. What dose-response relationship exists for dioxins and the most significant end points?
6. Can the problem of the exposure to dioxin mixtures be solved with respect to humans?
7. Can animal data help, when there are only suspicions for certain adverse effects?
8. Are the animal data consistent enough among species to allow extrapolations to humans?

ad **aspect** (**1**): Compared with many other "environmental substances", the situation with respect to dioxins is rather favorable, especially for TCDD. Within the last decade, many data were gathered on kinetics and on various biological or toxicological end points in humans.

ad **aspect** (**2**): Within the past decades, numerous measurements were performed on body burdens (mostly measuring concentrations related to body fat) of humans highly exposed to TCDD

and to other congeners, as well as of the general population. Therefore, we have an unusually large database available when compared with other environmental agents.

ad **aspect** (**3**): From animal as well as human studies, there might be four main toxicological endpoints of concern with respect to TCDD (besides chloracne): (a) alterations in reproductive and developmental variables, (b) effects on the immune system, (c) carcinogenicity, especially hepato-carcinogenicity, and (d) alterations in liver morphology and function. Because of the limited space available, only a few aspects are discussed here to illustrate the situation with respect to the various end points. Some considerations have been discussed elsewhere [1].

Some Aspects of altered DEVELOPMENT and REPRODUCTION:

The induction of cleft palate in mice was the main teratological finding as early as in 1971/72, reconfirmed over a period of 25 years in various laboratories. This effect can difficult or even not at all be reproduced in other animal species. Furthermore, comparatively high doses are required. Some *in-vitro* studies were performed, but the results are worthless for a human risk assessment. Only limited data on human pregnancy outcome are available. Due to the (fortunately) small number of pregnant women highly exposed to TCDD in Seveso, no increase in major malformations was found, but the relatively small number of mother/child-pairs limits far-reaching general conclusions. TCDD certainly is not a strong teratogen, neither for nonhuman primates nor for humans.

An intriguing result was observed after the Seveso accident: to the TCDD-exposed families, more girls than boys were born during a certain period after the accident [2]. After a period of 10 years, the gender ratio seemed to return to normal. Several explanations are possible. If directly TCDD-related, this would represent one of the most sensitive effects of dioxins in humans.

Some Aspects of CARCINOGENICITY:

Epidemiology, like most medical sciences, is incapable of verifying small effects ("borderline effects"). In such situations, inevitably contradictory results must be obtained in different studies. This also is typical for the data on TCDD exposure. There is no indication that TCDD is a strong carcinogen in humans, especially not for the end points revealed from animal studies. Interpretation of the results of several studies is complicated because possible effects after poly-exposure were investigated, TCDD being one of the minor components ("trace contaminant"). Individual body burdens in the deceased were not measured in any of the studies, although this was shown to be feasible [3]. Instead, individual exposure (or even any individual exposure at all) was always deduced from indirect evidence. Elaborate mathematics and daring assumptions were frequently used (e.g. extrapolation of “exposure indices” of doubtful value) to compensate for the lack of data on the exposure of the cancer cases. Important confounders, such as smoking, have not been taken into account, even when an association of TCDD with lung cancer was suggested. A recent reinvestigation on the largest group of exposed chemical workers [4] also is not of much help in clarifying the issue. While it is possible that subsequent to massive exposure TCDD is weakly carcinogenic, probably by promotion-like effects according to experimental studies, the current weight of evidence for its classification as clear-cut “human carcinogen” is meager, and statements made are more political than scientific. Especially the practice of lumping together all types of human tumor diseases, because only in this way statistical significance can be achieved, is highly debatable. No specific epidemiological information exists on other "dioxin" congeners, and

conclusions with respect to humans from the very few animal data available would be pure speculation.

The accurate dose-response of possible TCDD-induced effects in humans is largely unknown, as is the possible existence of polymorphisms. Since a biphasic effect may be assumed to exist according to animal data, it is quite feasible that small doses of TCDD may even be "anti-carcinogenic" with respect to some or even all tumor incidences in humans. Available human data are insufficient to substantiate such a clue from animal studies. Because the effect of massive exposures is small, at best, all available information argues against TCDD being a substantial "carcinogen" for the not especially exposed general population. There is no information on a possible potentiation of other carcinogenic effects (and which) by TCDD in humans.

Some recent data on IMMUNOLOGY:

Extensive animal data have been accumulated on effects of TCDD on the immune system [5,6,7]. Of special significance are results of studies with very small doses of various "dioxins" on nonhuman primates, because similar studies can be performed with an identical technique in highly TCDD-exposed humans. Data of animal studies may be either reconfirmed in humans, or the significance of animal data for humans can be proven to be irrelevant. It is worth mentioning that not all effects of TCDD reported in animal studies can be adequately studied in humans.

There are two effects induced by TCDD and other dioxins with high reproducibility in a nonhuman primate (*Callithrix jacchus*): (a) a dose-dependent reduction of helper-inducer cells

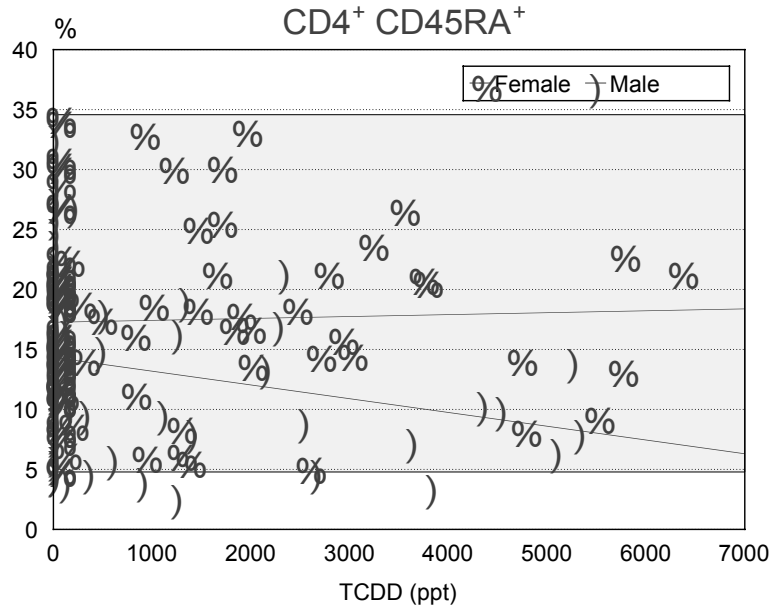

Figure 1 : Plot of the relative number of CD4+CD45RA+ cells in blood versus the measured TCDD body burden (in 1976) in adult volunteers from the Seveso area, Italy. Female (n = 102) and male (n = 94) volunteers are indicated. The plot is not compensated for age of the volunteers nor for other confounding factors. Age of the volunteers: 27 to 70 years.

("memory T cells) in peripheral blood, and (b) a reduction in B cells (CD20 cells). The first effect is reversed in this species at extremely low doses [8]. In some clean-up workers [9] and some residents of the Seveso area highly TCDD-exposed as children, a slight statistical significant increase in the average number of "memory helper T cells" ($CD4^+CD45R0^+CD45RA^-$) and a concomitant decrease in "naive" helper T cells ($CD4^+CD45R0^-CD45RA^+$) was observed, but almost all values stayed within the reference range.

Corresponding data obtained from residents 1976 exposed as adults with very high TCDD body burdens after the Seveso accident also did not provide any evidence for changes similar to those observed in TCDD-exposed nonhuman primates. This holds true when either considering the initial body burdens (i.e. analyzing the problem of persistent changes, *see:* figure 1), or when basing the data on the concurrent (i.e. the 1992/94 remaining) TCDD concentrations in blood fat. The individual TCDD body burdens were quantified in all of the volunteers, right after the accident and at the time of the immunological trial. There was never a TCDD-induced reduction in "memory helper T cells", and we never found any evidence for a reduced number of B cells, even when body burdens exceeded 1,000 ppt TCDD. As shown in figure 1, the apparent decrease in $CD4^+CD45R0^-CD45RA^+$ cells is observed in men, but not in women. This trend is statistically significant in group analyses and when evaluating a linear regression ($p = 0.023$), but with a very low regression coefficient (r^2[adjusted] = 4.5%). Despite a statistical significance, such a low regression coefficient is of no medical significance. Since age is a strong confounder, the trend shown disappears in a multi-regression analysis and the influence of the TCDD exposure becomes negligible in comparison to the age-induced reduction. Thus, the clear-cut effects observed with the same technology (flow cytometry and identical anti-human monoclonal antibodies) in all monkeys examined at extremely low doses (single dose of 10 ng TCDD/kg body weight) cannot be confirmed to occur in humans. The example shown stresses the necessity for documentation of individual data, the number of data points outside the reference range, and the importance of taking known strong confounders into account.

Another recent result was even more surprising. We analyzed the time course of the response to a tetanus vaccination. Normally, the proliferative response to a recall-antigen and the antibody titers decline with time after a booster with tetanus toxoid (figure 2, *see controls:* difference between first and second vaccination period). In a nonhuman primate we had seen some evidence for a delayed decline [10]. This result of an extended antibody response over a prolonged period was confirmed in volunteers who had been highly TCDD-exposed in Seveso (figure 2). The effect seems again more pronounced in men when compared with women, but only few women were studied.

Interestingly, this effect certainly cannot be designated as "adverse". If any, it might provide an advantage to the exposed person. Thus, certainly not all effects induced by TCDD can be considered as "toxic."

Some Evidence for increased incidences of HEART DISEASE and DIABETES:

In some studies an increased incidence of two additional end points have been reported: mortality of heart diseases and occurrence of diabetes. Again, the results of different studies are quite controversial. No convincing trend was found [4] in the most recent publication (SMR of 1.28, 95% CI = 0.92-1.72) with respect to deaths of heart diseases. If the trend were real, it remains an open question whether this would be a direct or an indirect effect of TCDD exposure.

Reports on an increased incidence of diabetes also show considerable weaknesses. If a diabetes type II was monitored, information on body mass indices would be essential, just to mention one aspect. Such information is lacking. Again, many explanations, besides a direct effect caused by TCDD or 2,4,5-T, are possible. Furthermore, both diagnoses mentioned were often made from death certificates, a source known to be unreliable for such a purpose. This was also critically remarked by some of the investigators.

ad **aspect** (**4**): Risk assessment from human data in the case of TCDD is complicated by the fact that exposure to dioxin was often associated in chemical workers with a hundred to several thousand times higher long-term exposure to other substances (TCDD being present as trace contaminant) at the work place. In such studies on poly-exposure it is daring to draw meaningful conclusions with respect to a single agent. Certainly, no possibility exists to obtain information on toxic effects of a single agent, when a mixture is applied. An effect observed may have been induced by any one of the agents in the mixture, or may have been caused by a special combination of them. Only with respect to a negative outcome, it could be concluded that no effect was induced by any of the agents at this dose level (antagonistic and agonistic effects of two agents occurring at the same time excluded). The situation is different at some acute accidents, such as in Seveso, because the short-term exposure was largely to TCDD.

ad **aspect** (**5**): The dose-response at very low doses of TCDD is quite obscure. Relying on the animal data, dose-dependent opposite effects on several end points cannot be dismissed, and in fact they have been experimentally demonstrated on important targets, such as some aspects of carcinogenicity and some immunologic variables [8,11]. Therefore, it is always daring to perform anything similar to a linear extrapolation of TCDD-induced effects to very low dose-levels, even in the same species. There is no scientific basis for such a quantitative extrapolation to humans.

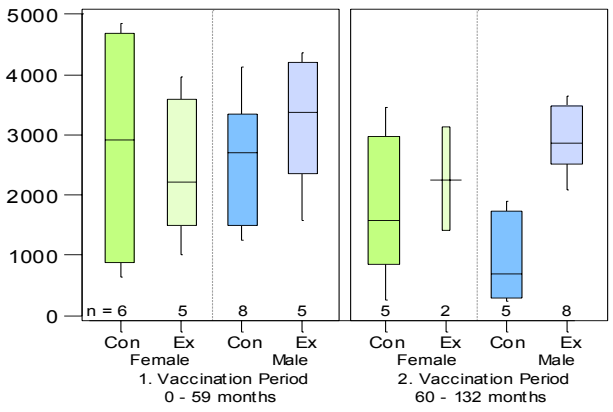

Figure 2. Proliferative response to tetanus toxoid (Δcpm) *ex vivo* using blood from volunteers TCDD-exposed in 1976 during the Seveso accident. Analysis performed in 1992/94. "Con": controls, "Ex": exposed persons regardless of the extent of exposure. Obvious is the clearly prolonged response in male volunteers during the second vaccination period, 60-132 months after the last booster.

ad **aspect** (**6**): Human exposure from combustion sources rarely occurs to a single "dioxin", but mostly to a mixture of several, or even numerous, congeners. Administrators and regulatory agencies have attempted to tackle this problem pragmatically by introducing the "International TCDD Toxicity Equivalency Factors" (I-TE factors). While this strategy may be acceptable and useful for preventive measures, there is no scientific basis for using such factors for quantitatively predicting toxicity of these congeners with unknown potency in humans. The suggestion of comparative I-TE factors refers strictly to rat studies, mostly performed at the high dose range, and for general signs of toxicity (preferentially chronic toxicity). Since the kinetics and

organ distribution of the “dioxins” in humans and animals are quite different, a wide range of comparative factors and of many confounders must be assumed to exist. However, it seems fair to state, without giving exact numbers, that the biologically highly active 2,3,7,8-substituted congeners should also be especially relevant to the situation in humans. Extending I-TE factors to the "dioxin-like" *non-ortho* PCB or even *mono-ortho* PCB, is pure speculation with respect to possible effects in humans. In this respect, it more confuses than clarifies the issue.

ad **aspects** (**7** + **8**): Generally speaking: there are agents and experimental situations, for which defined animal data may allow simple extrapolations to humans. Unfortunately with respect to toxicity, TCDD does not belong to this group of substances. This is predominantly due to species differences in kinetics of several orders of magnitude as well as to greatly differing tissue distributions. Any quantitative extrapolation between species is only meaningful when these kinetic aspects are taken into account. This difficulty is amplified when congeners other than TCDD are considered.

Furthermore, in many respects established effects of TCDD are not consistent among gender and species. This makes extrapolations to the situation in humans impossible (are humans comparable to male or female rats?), and usually "worst case assumptions" are made. Such considerations may be justified in regulatory toxicology (preventive hazard minimization), but they are without value when quantitatively predicting effects in humans. Thus, clinical toxicology again solely relies on human data. If such data are lacking, it is fair to clearly admit our ignorance in this respect.

Also considerable species differences exist with respect to some toxico-dynamics (e.g. effects on immunological end points, irrelevance of experimentally established thyroid or liver carcinogenicity for humans, species differences in the susceptibility for induction of hepatic monooxygenases). For these reasons, extrapolations to predict "safe levels" for humans from animals data have not been rewarding in the case of TCDD, and values differing by orders of magnitude have been suggested and "calculated" by different regulatory agencies.

Conclusions:

While providing some information on the organotropy and possible toxic potential of dioxins, animal data have not been helpful for quantitative comparative extrapolations of TCDD effects from animals to humans. The importance of animal research is largely in revealing mechanisms of TCDD actions, relevant to several animal species including man.

In group or regression analyses of medical data on human TCDD exposure, sometimes a statistically significant dose-dependent trend is indicated, but all data stay within the reference range (e.g. figure 1), and the regression coefficient is a few percent. Taken as such, these data do not represent a medical risk, although a deviation from the reference range cannot be excluded at higher exposure levels. However, no evidence for such an assumption can be provided.

The available human data on TCDD do not support the assumption that humans are especially susceptible to the several toxic actions of dioxins, although certain deviations from a reference range, often surrogate markers, may be observable in some exposed individuals [12]. In most investigations and with respect to all end points evaluated (perhaps with the exception of chloracne), the majority of individuals highly exposed exclusively to TCDD did not show any or only transient adverse health effects. This behavior of TCDD differs considerably from that of most other substances within a toxic dose range. The low susceptibility could partly be due to the

high proportion of body fat in humans compared with many experimental animals, and a considerable deposition of the highly lipophilic dioxins in this compartment [13], resulting in comparatively low target tissue concentrations.

Not all symptoms seen in humans may be directly caused by dioxins or other chemicals, and secondary e.g. psychological effects, such as fear and anxiety of unknown consequences of the exposure in persons known to have been exposed, including those with disfigurement, must be seriously considered.

Overall, the observed change in sex ratio [2] may turn out to be the most interesting effect.

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