SOME ASPECTS ON THE TOXICITY OF POLYHALOGENATED DIBENZO-P-DIOXINS AND DIBENZOFURANS IN ANIMALS AND MAN

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INTRODUCTION

"Dioxins" are modern (X 1989). The name is known to almost every citizen and almost no day passes on which there is no mention of these substances in newspapers and journals. Despite the popularity of these substances there are not too many scientists who are competently able to judge the possible adverse health effects induced by these substances and even fewer can do so on the basis of extended, personal experience.

While the general principles of pharmacology and toxicology, e.g. dose-effect relationships, also apply for these classes of substances there seem to be a number of special aspects which are not that easily understood.

It is impossible to give a detailed overview on the toxicity of PHDDs/PHDFs in this brief presentation, but a comprehensive compilation of the most relevant data has recently been published, including an extensive citation of the literature (WHO 1989). Some of the special features of the toxicity of polyhalogenated dibenzo-*p*-dioxins and dibenzofurans will be discussed. The following aspects will be included:

- Selection of the substances with high toxicological significance;
- What does "toxic" mean with relevance to man ?
- Toxicity of high doses vs. biological effects of low doses;
- Toxicokinetic characteristics of PCDDs/PCDFs;
- Concept of no-observed-adverse-effect-concentration (NOAEC) or: Basing toxicity on the corresponding body burden;
- Is the toxicity of PCDDs/PCDFs receptor-mediated ?
- Some special aspects on the toxicity of PCDDs/PCDFs
  - Problem of increase of tumor incidence,
  - Problem of prenatal toxicity,
  - Problem of possible immunotoxicity;
- Data on other polyhalogenated dibenzo-*p*-dioxins and dibenzofurans;
- Some remarks on possible dioxin-induced toxic effects in man.

1. Selection of the substances with high toxicological significance

Numerous polyhalogenated dibenzo-*p*-dioxins and dibenzofurans may be formed and many have been found in the environment. Besides the 210 possible chlorinated substances, the same number of brominated congeners may exist. From the mixed-halogenated congeners, containing chlorine and bromine in the same molecule, several thousand substances are theoretically possible. At first glance it, therefore, may seem a hopeless task to discuss the toxicity of all these substances. Fortunately, not all of them seem to occur in the environment, and even more important: not all of them have the same toxicological significance.

Besides the dose, pharmacological and toxic effects always depend on kinetic as well as dynamic aspects of the substance in question. Therefore, these characteristics are also the crucial ones when attempting to evaluate the possible toxicological significance of PHDDs/PHDFs.

ABBREVIATIONS

TCDD	=	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin;
TBrDD	=	2,3,7,8-Tetrabromodibenzo-*p*-dioxin;
PCDDs/PCDFs	=	Polychlorinated dibenzo-*p*-dioxins and dibenzofurans;
PHDDs/PHDFs	=	Polyhalogenated dibenzo-*p*-dioxins and dibenzofurans;
EROD	=	Ethoxyresorufin O-deethylase

From the large number of congeners those are of special interest:
- which are persistent in the environment and accumulate in the mammalian organism,
- which have a high toxic potency, and
- to which a significant exposure occurs.

For various reasons these are the 2,3,7,8-substituted congeners. The non-2,3,7,8-substituted congeners largely do not persist in the mammalian organism (Abraham et al. 1989; Neubert et al. 1990a) and do not lead to accumulation. Furthermore, they exhibit a much lower biological potency than the 2,3,7,8-substituted isomers. Therefore, for practical toxicological considerations the relevant number of congeners is most often reduced to only about 10% or less (namely the 2,3,7,8-substituted ones) of all the polychlorinated and polybrominated substances in a mixture.

2. What does "toxic" mean with relevance to man ?

As is the case with many medical idioms, the term "toxic" is difficult to define and may be misleading to the layman. With relevance to man "toxic" refers to effects adverse to human health, and therefore, scientifically it cannot be associated with a substance but only with a dose or an extent of exposure. Adverse health effect means clearly demonstrable subjective or objective signs of distress or dysfunction of the mammalian (and preferrentially human) organism.

When a pronounced adverse health effect results from the action of a substance it is easy to agree that this is toxic. However, as is well-known to every medically-trained person, a broad borderline or "gray" zone exists in which an adverse effect may be relative. The effect of a thyreostatic drug is, e.g., considered "beneficial" when given as a medication to a patient with hyperthyreoidism, but a thyreostatic effect will be considered "adverse" if produced by a sufficient exposure to a herbicide. A similar situation exists when attempting to define adverse effects of, e.g., atropin at therapeutic doses. We have to accept that not every biological effect of a substance can be called "toxic", i.e adverse to human health. Otherwise every pharmacological and biological action would be toxic.

Such an example is the enzyme induction of hepatic monooxygenase activities caused by many drugs and environmental chemicals. This is also clearly a biological effect caused by the substance, but it can, as such, not be considered "toxic", especially not if the enzyme activity is only slightly altered.

For environmental pollutants without any benefit to man the level of an acceptable exposure may be different: not only exposures leading to clearly "adverse" health effects, but already those inducing any detectable biological action in man, may be considered undesirable or unacceptable.

Furthermore, it is often not recognized that quite a different problem exists when:
- possible adverse health effects after an exposure are to be medically evaluated, or
- relevant exposures of a population are to be prevented as a precautionary (often purely political) measure (e.g. by defining "virtually safe dose levels").

3. Toxicity of high doses vs. biological effects of low doses

It is difficult to briefly summarize the various biological and toxic effects of PCDDs/PCDFs as evaluated in different species. Therefore, the following considerations should mainly refer to effects as observed in rodents, as the best-studied species. Some comparisons with effects seen in non-human primates and in man will be made.

Effects which may be considered clearly toxic, such as weight loss or thymus involution, may be triggered by TCDD in susceptible species at single doses of the lower µg/kg-body wt-range (cf. Table 1). This dose range has so far been preferred in the majority of studies with TCDD. The dose level is about three orders of magnitude higher than the doses capable of triggering just detectable hepatic enzyme inductions.

No data are available on more subtle but clear-cut toxic effects of TCDD at lower doses (hundreds of ng/kg body wt). In this respect the behaviour of PCDDs/PCDFs very much resembles that of many other polychlorinated substances, such as DDT, HCHs and PCBs, for which also a wide span exists between the first detectable biological effects and the occurrence of clearly toxic symptoms.

As mentioned before, all of these substances have the potency to induce hepatic monooxygenases (at least in experimental animals). With the most potent PCDDs/PCDFs this enzyme induction may be just detectable in rodents subsequent to dosing in the lower ng/kg-body wt-range, leading to additional hepatic tissue concentrations in the lower ppt-range. Similar results have been obtained in non-human primates (Krüger et al. 1990; Schulz-Schalge et al. 1990b). At this dose- and concentration-range no obvious "toxic" effects are detectable.

The phenomenon of enzyme induction of hepatic monooxygenases is demonstrable for many classes of polyhalogenated aromatic substances with one or more ring systems. Such substances include: DDT, HCHs, HCB, PCBs and PCDDs/PCDFs. These substances are very lipophilic and most of them are persistent in the mammalian organism. Therefore, when brought into the environment they will persist for many decades and will, after some time, be ubiquitously present, also (mostly in the adipose tissue) in man. This is the main reason for toxicological concern with respect to these classes of chemicals.

Some special aspects of toxicity will be discussed in chapter 7.

Table 1: Effects reported after a single dose of TCDD in rodents

porphyria	rat	>	10,000	ng/kg body wt
cleft palates	mouse	>	10,000	ng/kg body wt
thymus involution	rat	>	1,000	ng/kg body wt
immunotoxicity	rat	>	1,000	ng/kg body wt
myelotoxicity	mouse		1,000	ng/kg body wt
liver enlargement	rat	>	1,000	ng/kg body wt
liver impairment	rat	>	1,000	ng/kg body wt
on fertility	rat	>	1,000	ng/kg body wt
testes changes	rat	>	600	ng/kg body wt
EROD induction, liver	rat		1-3	ng/kg body wt

Corresponding max. concentrations (fat) (e.g. Abraham et al. 1988) subsequent to:

1	ng/kg body wt	about	3	ppt
10	ng/kg body wt	about	50	ppt
300	ng/kg body wt	about	800	ppt
1000	ng/kg body wt	about	2000	ppt

4. Toxicokinetic characteristics of PCDDs/PCDFs

For an assessment of the toxic potency of a "dioxin", information on kinetic variables is essential, and preferentially, data on the target tissue concentrations as well. Such kinetic data are rather well-established for the rat and reasonable data exist for some of the congeners in non-human primates (Neubert et al. 1990a; Hagenmaier et al. 1990). While in such animals data on tissue distribution and possible body burden have been collected over the past years, the knowledge of kinetic data for man is confined to casuistic information. Since a number of highly exposed populations exist (at least against TCDD) more data may be expected within the next years.

Many experimental studies indicate that the enteral absorption of 2,3,7,8-TCDD is > 80% if the substance is applied in a freely available form (Poiger and Schlatter 1980). Such a high rate of uptake is not guaranteed subsequent to an oral administration for some of the other congeners, especially not for the highly-halogenated ones.

As a consequence of this, exposure to a mixture of PCDDs/PCDFs through the oral route may be expected to lead to an uptake into the organism which is not identical with the composition of the exposure mixture, the tetra- and penta-chlorinated PCDDs/PCDFs being more readily absorbed (Hagenmaier et al. 1990). Although solid data are still lacking such a situation may also be expected in man, e.g. for an exposure via mother's milk. Such a differentiated behaviour is suggested from studies in monkeys (Hagenmaier et al. 1990). Therefore, it is likely that quantitative differences in the percentage of absorption and consequently in the extent of contribution to a biological action will show up when mixtures of PCDDs/PCDFs are applied by different routes. A higher rate of absorption is, e.g., feasible for highly-chlorinated congeners by the parenteral or the inhalative route when compared with oral administration. However, such differences are of minor significance when target organ concentrations are monitored during experimental studies or the body burden is assessed in human exposures. This, today, is an absolute must for experimental studies and is also quite possible when assessing human exposure. Tissue concentrations were not found to vary greatly when TCDD was administered by different routes, but differences in local manifestations of toxic effects may of course occur with different types of exposures (skin, lung, etc.).

Interestingly, it is reasonably well-established that very similar maximum tissue concentrations are obtained in different animal species (including man) after single moderately high doses of TCDD (Abraham et al. 1988; 1989; Poiger and Schlatter 1986).

With the exception of 2,3,7,8-TCDF and possibly 1,2,3,7,8-P5CDF all the 2,3,7,8-substituted PCDDs/PCDFs are persistent in the mammalian organism. The elimination half-lives may vary among species and the different congeners. This persistence, of course, leads to an accumulation when constant doses are applied daily over prolonged periods. According to simple pharmacokinetic rules, a steady-state situation is expected to occur. This has been confirmed in animal studies. Because of the large differences in the rates of elimination among species, long-term exposure will quantitatively lead to pronounced species differences, and considerable modifications in the dosing schedule are required when attempting to compare effects among species after multiple dosing. A long-term constant daily dose in the rat of, e.g., 1 ng TCDD/kg body wt may correspond to about daily 10 pg TCDD/kg body wt in man under steady-state conditions

(which are, of course, reached much earlier in the rat). These differences must be taken into consideration in the experimental design and for a risk assessment.

In the case of 2,3,7,8-TCDD such a maximum accumulation (at steady-state) is expected (with the assumed half-lives in paranthesis) for:

- the rat	($t/2 \approx$ 3 weeks,	*Rose et al. 1976*)	at the	30 -fold
- *Callithrix*	($t/2 \approx$ 10 weeks,	*Neubert et al. 1990a*)	at the	100 -fold
- man	($t/2 \approx$ 7 years,	*Poiger and Schlatter 1986*)	at the	3700 -fold

of the same single daily dose. Consequently, in man a single dose of, e.g., about 4 ng TCDD/kg body wt should result in the same body burden as a long-term daily exposure to 1 pg/kg body wt at the steady-state.

5. Concept of NOAEC or: Basing toxicity on the corresponding body burden

When the elimination half-life of a given substance is very different in the experimental species and in man, in all pharmacological and toxicological considerations comparisons of effects and extrapolations between species should no longer be attempted on the basis of dose levels. This also applies for any extrapolation of toxic effects of PCDDs/PCDFs between species. The kinetic aspects of PCDDs/PCDFs are now rather well-known in several species, including the rat and some non-human primates, and tissue concentrations have been assessed to some extent in man. Therefore, a comparison of effects between species should rather be performed on the basis of tissue concentrations. In this way even large species differences in kinetics may be compensated for. Remaining species differences will then solely be due to variations in the susceptibility of various species to the action of the substance at the cellular level.

On the basis of no-observed-adverse-effect-concentrations (NOAEC), preferentially in target tissue, a much better risk assessment may be achieved. Some NOAEC may be derived from the data compiled in Table 2. According to the data of Kociba et al. (1978) and of Pitot et al. (1987) the NOAEC (TCDD) for the induction of liver adenomas would be > 1,000 ppt in the target tissue. If every *biological* (not toxic) effect is considered (e.g. induction of hepatic EROD activity) the NOEC (TCDD) would be 5 to 10 ppt in the female rat (Abraham et al. 1988), and only slightly higher values were found in marmosets (Krüger et al. 1990; Neubert et al. 1990a). This concept will be more extensively discussed elsewhere (Neubert 1990).

6. Is the toxicity of PCDDs/PCDFs receptor-mediated ?

Several years ago Poland and Glover (1980) put forth the hypothesis that the toxic effects of TCDD may be mediated through stimulation of a defined receptor (TCDD-receptor). The evidence that TCDD reacts in a hormone-like fashion with a defined receptor has since been substantiated, and there is hardly a pollutant for which this mode of action (binding to the receptor, transport of the complex to the cell nucleus, interaction with defined DNA sequences, induction of certain biological effects) has been better studied. However, the crucial question remains whether this well-studied interaction is the only interaction TCDD exhibits with defined structures and functions of the mammalian organism, and secondly whether the toxic effects seen at comparatively **high** doses are the result of such a mechanism. There are still no good arguments for such a correlation, and the evidence that all symptoms produced by doses of e.g. > 10 µg TCDD/kg body wt in the rat are mediated through a TCDD-receptor are still more than meager. However, it should be remembered that at highly toxic doses of any substance so many processes within an organism are affected that the mechanisms of action can hardly be evaluated, and generally such analyses are not even considered worth attempting.

From the information available it is feasible that acute or chronic effects induced by rather low doses (lower ng/kg-body wt-range), leading to tissue concentrations at the 100- to 1,000-ppt-level, such as changes in lymphocyte subpopulations in certain non-human primates or tumor promotion in rodents may involve the help of a TCDD-receptor. Such changes induced by PCDDs/PCDFs resemble hormone actions in some respects, and the active concentrations are comparable with hormone concentrations and some doses required to induce well-known effects in man (e.g. 500 ng ethinylestradiol/kg body wt is a highly effective contraceptive dose in women).

7. Some special aspects on the toxicity of PCDDs/PCDFs

There are a number of aspects on the toxicity of PHDDs/PHDFs which deserve special attention because of their possible significance as serious adverse effects, special medical problems, or public concern.

7.1 Problem of increase of tumor incidence

The present situation with respect to an assessment of a possible carcinogenic potential of PCDDs/PCDFs is similar to that summarized three years ago (Neubert and Meister 1987). However, some new and important data must be discussed. It is noteworthy that the histological slides of Kociba et al. (1978) have recently been reevaluated and lower tumor incidences were found (Keenan et al. 1990, Dioxin '90, 1:549-554).

In studies in rodents (Kociba et al. 1978; NTP 1982) 2,3,7,8-TCDD was doubtlessly able to increase the incidence of liver tumors (adenomas) at daily doses of 10 ng/kg body wt (carcinoma were only seen at the high and hepatotoxic dose of 100 ng/kg body wt). This ability is shared by many polyhalogenated substances with aromatic ring systems (PCBs, HCHs, HCB, etc.) which have the capacity to induce hepatic monooxygenases and liver growth. This phenomenon has extensively been studied by the group of Schulte-Hermann (e.g. Schulte-Hermann 1974; Schulte-Hermann et al. 1983).

All of these substances show a variety of characteristics that distinguish them from the usual initiating carcinogens. These include:

- as TCDD they do not form adducts with the DNA (Poland and Glover 1979; Randerath et al. 1989), and they have little, if any, mutagenic potency (epigenetic carcinogenesis);
- they are inducers of hepatic monooxygenases and of liver growth in rodents (TCDD is the most potent one: Kitchin and Woods 1979; Abraham et al. 1988);
- *in vitro* they show (in malignant transformation assays) no initiating properties, but are potent "promoters" like TCDD (e.g. Abernethy et al. 1985);
- this "promoting" action of TCDD shows a clear-cut threshold (Pitot et al. 1987);
- at lower concentrations "protective" effects against tumor induction were noted (Pitot et al. 1987);
- TCDD exhibits, at the same dose, anticarcinogenic properties against several spontaneous (endocrine?) tumors (Kociba et al. 1978).

In the studies on tumor promotion (Pitot et al. 1987) a threshold was found at 10 ng TCDD/kg body wt per day. This is a similar NOAEL as was found in the long-term studies (Kociba et al. 1978; NTP 1982). In these important studies of Pitot et al. (1987) the diethylnitrosamine-induced tumor frequency was reduced at doses of TCDD lower than 10 ng TCDD/kg body wt. No initiating activity was found with respect to appearance of foci in the liver and their growth (volume percentage).

When testing a mixture of two hexa-chlorinated dibenzodioxins in a long-term carcinogenicity study, only liver tumors were observed in rats and mice (NTP 1980).

For the reasons mentioned the increase in liver tumor incidence in rodents should be considered a very special case. Extrapolations of such data with the usual mathematical models assuming no threshold is certainly unacceptable and without any scientific justification. It is questionable whether this special effect observed on the rodent liver is of any significance for man, at the usual level of exposure of our population. Primary hepatocellular carcinoma are extremely seldom in the populations of industrial countries.

7.2 Problem of prenatal toxicity

Data on prenatal toxicity of TCDD have been reviewed before (e.g. Neubert et al. 1990b). Here only a few aspects will be considered.

Teratogenic effects of TCDD (and of other congeners) have only been observed at rather high doses in the mouse, especially in the form of cleft palates (Courtney and Moore 1971; Neubert and Dillmann 1972; Neubert et al. 1973). Recently, the effect of TCDD on palate development was also confirmed in *in vitro* studies, and an involvement of EGF receptors and inhibition of programmed cell death were suggested as possible mechanisms of action (Abbott and Birnbaum 1989).

In several studies in non-human primates no teratogenic effects were observed (Allen et al. 1977; McConnell et al. 1978; McNulty 1985; Krowke and Neubert 1988). However, the data available from rhesus monkeys (McNulty 1984, 1985) and marmosets (Krowke and Neubert 1988) suggest that embryofeto-mortality may result from total doses exceeding 1,000 ng TCDD/kg body wt (given over a period of several months to years). In cases of surviving fetuses tissue concentrations measured were in the range of 6,000 to 10,000 pg TCDD/g adipose tissue (ppt) in the maternal organism.

There has been considerable controversy on a multigeneration study published by Murray et al. (1979). The authors considered a daily dose of 1 ng TCDD/kg body wt as the NOAEL. This was questioned on a statistical basis for some minor effects. In our opinion the apparent adverse effects were overinterpreted by the critic. It is well-known to any experienced investigator that minor variations among groups are bound to occur in extremely complex and long-term studies such as the one mentioned. Such deviations from the controls are normally not considered substance-related (in accordance with the interpretation of the authors). Nevertheless, it seems necessary to repeat the study to solve the controversy. Furthermore, the study, at that time, had not been coupled with kinetic investigations which would be necessary according to today's standard, since considerable variations in tissue concentrations of TCDD are to be expected during such a study (Krowke 1986).

No convincing or well-documented data have been presented on malformations induced in man by PCDDs/PCDFs. However, the predictive value of epidemiologic studies is often overestimated, and it is extremely difficult to detect xenobiotica-induced increases in spontaneous incidences of a low magnitude. Long-term studies on the population living in contaminated areas in Seveso had the resolving power to exclude a 2-fold increase in the overall rate of malformations and of some important single deviations in zone A and a 5-fold increase in zones AB, and they did not reveal such adverse effects (Mastroiacovo et al. 1988). Similarly, no significant increase in adverse reproductive outcome was found in women living in a contaminated area in Missouri (Stockbauer et al. 1988). However, of course, a small increase in rare abnormalities can never be excluded, although this also does not mean that this would be of great medical significance. In several reports the results of carefully conducted observations have been published on children of *yucheng* victims who were heavily exposed to polychlorinated biphenyls and their contaminants *in utero* (e.g. Rogan et al. 1988). A significant delay of developmental milestones was found, besides abnormalities of gingiva, nails, teeth and coloured skin. The authors suggested that much of the toxicity seen may be due at least in part to the contaminating PCDFs.

Table 2: Some effects reported after multiple doses of TCDD in rodents

			effect observed:		
			no	slight	pronounced
			ng/kg body wt. per day		
TCDD					
liver tumors	*(Kociba et al. 1978)*	rat	1	10*	100
multigeneration study	*(Murray et al. 1979)*	rat	? 1	10	100
porphyria	*(Cantoni et al. 1981)*	rat	14		143
123678-/123789-H6CDDs					
liver tumors **	*(NTP 1980)*	rat	180		360
	(NTP 1980)	mice	360		715

* no carcinoma; ** liver tumors only observed;

Corresponding concentrations at steady-state (e.g. Kociba et al. 1978) subsequent to:

1	*ng/kg body wt*	*about*	*540*	*ppt*	*(liver)*
1	*ng/kg body wt*	*about*	*540*	*ppt*	*(adipose tissue)*
10	*ng/kg body wt*	*about*	*5,100*	*ppt*	*(liver)*
10	*ng/kg body wt*	*about*	*1,700*	*ppt*	*(adipose tissue)*
100	*ng/kg body wt*	*about*	*24,000*	*ppt*	*(liver)*
100	*ng/kg body wt*	*about*	*8,100*	*ppt*	*(adipose tissue)*

7.3 Problem of possible immunotoxicity

There is no doubt that, e.g. in rodents, relatively high doses of TCDD (> 5 µg/kg body wt) are able to interfere with several functions of the immune system, when assessed in several test systems (e.g. Vos 1977; Vos this meeting). As mentioned before these doses have to be considered highly toxic, also with respect to other actions. It is much more difficult to answer the question whether signs of immunotoxicity may also be detectable at rather low levels of TCDD (i.e. at the hundreds of ppt level in major organs, or subsequent to single doses in the ng/kg-body wt-range). No data have been reported on allergic reactions caused by TCDD. Much more immunological information is required in experimental animals and man.

There are only two reports in the literature on possible effects of low doses of TCDD on functions of the immune system. It is questionable whether the effects observed must be considered adverse health effects, rather than a biological variation. The first clue for a possible effect of TCDD was reported by Clark et al. (1981; 1983) in an *in vitro/in vivo* system after treatment of mice with multiple doses of 4 ng TCDD/kg body wt, or even lower. Unfortunately, this finding has not been confirmed in another laboratory.

The second report comes from our group. Administering single doses (s.c.) of TCDD to a non-human primate (*Callithrix jacchus*) and analysing peripheral venous lymphocytes with monoclonal antibodies and flow cytometry (FACScan-technique) we could demonstrate a reduction in the percentage of the $CD4^+CDw29^+$ (T cell-) subpopulation and in the $CD20^+$ (B cell-) population subsequent to doses as low as 10 ng TCDD/kg body wt (Neubert et al. 1989; 1990e). No significant effect was seen following a dose of 3 ng TCDD/kg body wt. Although the "helper-inducer" subpopulation affected ($CD4^+CDw29^+$), which have also been designated as "memory cells", may play an important role in immunological processes, again, the

medical significance of such a change in the percentage of lymphocyte subpopulations is questionable. However, it certainly is a biological variation induced by an extremely low dose of TCDD. The lymphatic system as well as the bone marrow of this non-human primate seems to be especially sensitive to the action of TCDD, since a single dose of 1,000 ng/kg body wt proved to be generally haematotoxic.

Further *in vitro* studies revealed that in addition to the well-known effect on the thymus (Greenlee et al. 1985), also a direct effect of TCDD on peripheral lymphocytes is feasible (mixed lymphocyte culture). Such an effect may be observed *in vitro* with concentrations as low as 10^{-13} to 10^{-15} M TCDD (Neubert et al. 1990d). Again cells with the epitopes: $CD4^+CDw29^+$ and $CD\ 20^+$ were predominantly affected. This correlated with a reduction of the number of cells developing IgG lambda and kappa chains on their surface during culturing. Human lymphocytes exhibited the same susceptibility to this *in vitro* action of TCDD as corresponding cells from marmosets.

There is no convincing evidence for effects on the immune system by TCDD in man (e.g. studies in Seveso; Hoffman et al. 1986; Evans et al. 1988), but the monitoring has not been performed up till now with modern and sensitive techniques. In *yucheng* victims with a combined exposure to PCBs and PCDFs changes in some functions of the immune system have been reported.

8. Data on some other polyhalogenated dibenzo-p-dioxins and dibenzofurans

No lengthy consideration will be given to the problem of "TCDD-toxic-equivalency" factors since these aspects have been discussed before (Neubert et al. 1990c). However, it should be remembered that TE-factors represent no more than pragmatic means for regulatory purposes, and they have even been included in the laws of some countries. Their scientific value and their significance for a specific medical risk assessment is very small, if at all existent. This is derived from the fact that comparisons of various effects of several substances between different species may only be attempted when dose-response curves are available and pharmacokinetic variables are known. Since there are good indications that such dose-response curves will not run parallel in the case of PCDDs/PCDFs, different "TE" ratios are bound to result for a given congener when various biological endpoints, different species and different dose ranges (low vs. high doses) are considered. For this reason TE-factors must scientifically remain unjustified compromises. Up till now, no data have been presented for the multiple dose-response relationships mentioned even for a single pair of substances from these classes.

In this short overview it is impossible to discuss the toxicities and toxicokinetics of all of the remaining PCDDs/PCDFs. Among others, work from the laboratories of Poiger and Schlatter, from Birnbaum, and from our own group have largely contributed to compiling information in this field. Furthermore, the area is even more extended since some co-planar PCBs (as also discussed in this meeting) seem to exhibit similar toxic properties as the "dioxins".

Besides TCDD, 2,3,7,8-TCDF has been rather well studied in rodents and non-human primates. Recently a controversy has arisen on the toxicity of OCDD. This congener, occurring at comparatively high concentrations in human fat, had so far been assumed to exhibit a very low toxicity, and a toxicity equivalency factor of 1/1000 of that of TCDD was assigned. Data on rats from Couture et al. (1988) obtained in the high-effect-range might suggest a somewhat higher toxicity of OCDD than anticipated. A toxicity between 1/300 to 1/1000 as compared with OCDD is suggested from recent studies of Poiger and Schlatter (1990, this meeting). However, our own studies on the hepatic EROD induction in the same species at the low-effect-range suggest an at least 1/1000 lower potency of OCDD than TCDD when compared on the basis of tissue levels (Golor et al. 1990; Neubert et al. 1990c).

While abundant toxicological and kinetic data on PCDDs/PCDFs have been published, although not to the same extent for all of the congeners, information on the toxicity of *other* PHDDs/ PHDFs is rather scarce.

Some data have become available from a 90-day toxicity study with 2,3,7,8-TBrDD (Löser and Ivens 1989) in Wistar rats, suggesting a 3- to 10-fold lower toxicity when compared to TCDD, but with symptoms comparable to those known from the action of TCDD. The LD_{50} seemed to be between 3 and 10 µg/kg body wt, and only daily doses of 100 ng/kg or less did not lead to a reduced weight gain or a clear-cut reduction of the relative thymus weight. Triiodothyronine and thyroxine levels in serum increased and decreased correspondingly subsequent to daily doses of > 10 ng/kg body wt. Since up till now no data on the pharmacokinetics have been reported, it can presently not be ruled out that the proposed difference in potency between TBrDD and TCDD may be due to differences in absorption.

Studies on the potency of induction of hepatic EROD activity in rats (Nagao et al. 1990a) subsequent to low subcutaneous doses of TBrDD (< 1 µg/kg body wt), and of the cleft palate-inducing potency of this brominated congener (Nagao et al. 1990b) point to a rather similar potency of TBrDD and TCDD. In all of these studies with single doses no higher potency of the brominated congener was found on a molar basis, but the kinetics seem to be different, i.e. the brominated congener was more persistent in adipose tissue.

Recently preliminary data have become available (Schulz-Schalge et al. 1990a) on the potency of mixed-halogenated congeners, i.e. those containing chlorine and bromine in the same molecule, to induce hepatic EROD activity. Subsequent to a single dose none of the three tested 2,3,7,8-tetra-halogenated congeners was more active than 2,3,7,8-TCDD on a molar basis. Similarly, none of the tested tetra-halogenated congeners was more active than TCDD *in vitro* in inducing EROD activity in hepatocytes (Blankenburg et al. 1990). However, no data on the kinetic behaviour or tissue distribution are yet available.

9. Some remarks on possible dioxin-induced toxic effects in man

The major difficulty in assessing possible toxic effects induced by TCDD and its congeners in man has long been a reasonable definition of the exposure. Most often only an exposure against herbicides, pesticides or industrial chemicals (possibly contaminated by PCDDs/PCDFs) has been documented. Although the extent of exposure to PCDDs/PCDFs (if any) was impossible to judge, conclusions with respect to toxic effects hypothesized to be induced by just these substances were drawn. This includes studies on a possible causal relationship between such ill-defined exposures (e.g. of farm or industrial workers) and an increased tumor incidence, and such reports have largely complicated an assessment of possible toxic effects by TCDD and similar substances when (some often questionable) correlations were reported. Such results from ill-defined mixed exposures will only allow some conclusions with respect to dioxins when a "negative" outcome is found. Otherwise it can only be concluded that farmers or industrial workers exposed to a variety of chemicals show a rate of abnormality different from that of a control population, which often may not even be directly comparable. Therefore, at today's scientific standard no epidemiological studies should be performed without an attempt to define the type and quantity of exposure to PCDDs/PCDFs, or no conclusions with respect to possible effects of "dioxins" should be drawn from such studies. Furthermore, grant-giving institutions should take care that a proper peer review of the protocols and an accompanying expert group is at hand to avoid the recently created data cemeteries and false claims in this area, e.g. recently established in Germany, and to avoid the wasting of tax money.

Table 3: Comparison of effects induced by TCDD in animals and reported in man

	in animals	in man
hyperkeratosis	in monkeys, hairless mice	typical and frequent symptom
monooxygenase induction	typical effect	little evidence
liver damage	at very high doses	at very high exposures ?
porphyria	at very high doses	little evidence
hypercholesterinaemia	little evidence	typical symptom ?
neurological symptoms	no evidence	typical symptom ?, causality questionable
behavioural symptoms	no evidence	typical symptom ?, causality questionable
immunological effects	at high doses	little, if any, evidence
malformations	only in mice at very high doses	no convincing evidence
liver tumors	in rats and mice	little evidence
other tumors	with TCDD at high doses ?*	little evidence

* not with hexa-chlorinated CDDs

A comparison of some of the symptoms reported in man and effects observed in rodents is given in Table 3.

Most epidemiologic medical studies conducted so far in this field also suffered from a limited control of confounding factors and they had a rather limited resolving power. An overview on such studies was given by Fingerhut et al. (1987). In well-planned, well-conducted, and well-documented studies little evidence was obtained for an increased rate of tumors in exposed people. Many of these studies did not show a significant increase in the rate of defined tumors when compared with controls (Zack and Suskind 1980; Bertazzi et al. 1989; Zober et al. 1990), and it was concluded that a cancerogenic risk for man induced by TCDD even at high exposures may be expected to be low, if present at all. Further critical studies will be necessary to decide whether TCDD has any significant cancerogenic potency in man or not.

Thus, there is only little information available on adverse health effects in man observed after a "clean" exposure to TCDD or similarly defined substances. When evaluating the few examples of a rather defined exposure, a similar situation as discussed for experimental animals seems to exist with respect to man. Except for extremely high exposures, many orders of magnitude above an exposure of the general

population, very few (if any) clear-cut toxic effects have been reported subsequent to "clean" exposures to PCDDs/PCDFs. Even at extreme exposures against TCDD (like that of some residents in Seveso) clear symptoms of toxicity (except for chloracne in children) were absent or at least difficult to recognize. Also variables of laboratory medicine observed in children over a period of six years were not abnormal, and confined in some of the cases to γ-glutamyltransferase and aminotransferase activities inside limits set from the end of the normal range to slightly above it, and the changes disappeared with time (Mocarelli et al. 1986), and to an apparent increase in urinary D-glucaric acid excretion in some of the children (Ideo et al. 1982; 1985). Retrospectively, it was found that some of the children with chloracne had accumulated concentrations > 10,000 ppt in the fat of blood plasma. These are among the highest concentrations ever measured in man, and they exceed tissue levels in the normal population by a factor of 1,000 to 10,000. There are many more examples of humans with high exposures against TCDD, and those carrying body burdens at the ppb level in the fat without obvious adverse health effects (e.g. Facchetti et al. 1981; Young 1984).

From these observations it is tempting to conclude that man does not belong to the especially sensitive species against TCDD, and uncertainties in diagnosing clear-cut symptoms have been discussed (Webb et al. 1986). These conclusions seem to be in contrast to the numerous symptoms described in workers having been subjected to mixed exposures (including PCDDs/PCDFs) and those of the Yusho and Yucheng cases. If one is not willing to assume that some of the PCDFs or higher chlorinated dibenzodioxins possess other toxic properties than TCDD, many of the symptoms described may not have been caused by TCDD (alone), but by other chemicals present in the exposure mixtures. However, special susceptibilites of some individuals towards TCDD cannot be excluded at present.

It has become modern among some self-elected demagogic "experts" in our country (for definition of an "expert" cf. Hutzinger, 1989) and irresponsible journalists to deliberately spread wrong information and to relate all kinds of observations or even claimed adverse effects (such as all types of cancer, birth defects, allergies, etc.) which occur in a given area to "dioxins", if such substances have been detected at any level in the larger surroundings. For some people "dioxins" have taken up the position of the devil from the middle ages. It is often forgotten that any kind of toxicity is a matter of dose or extent of exposure. One wonders whether more adverse effects have been induced in our population by the told or published horror stories than directly result from PCDDs/PCDFs. There is no doubt that pollutants such as PCDDs/PCDFs, which have no usefulness, have to be removed from the environment as much as possible. However, in the long run no benefit will result from exaggerations and false claims since they will prevent these pollutants from being given their rightful place among toxic substances and their due consideration in our environment.

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