EXECUTIVE SUMMARY

Assessment of the health risk of dioxins: re-evaluation of the Tolerable Daily Intake (TDI)

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INTRODUCTION

Polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) constitute a group of persistent environmental chemicals. A number of dioxin or furan congeners, as well as some co-planar PCBs have been shown to exert a number of toxic responses similar to those of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), the most toxic dioxin. These effects include dermal toxicity, immunotoxicity, reproductive effects and teratogenicity, endocrine disruption and carcinogenicity.

During the last years the WHO European Centre for Environment and Health (WHO-ECEH) has been coordinating a comprehensive programme in collaboration with the International Programme on Chemical Safety (IPCS) on PCDDs, PCDFs and PCBs, aiming at evaluating the possible health risk, and prevention and control of environmental exposure of the general population to these chemicals.

Several WHO meetings in the field of the health risk assessment of dioxins and related compounds have been convened. At a meeting held in Bilthoven, The Netherlands (December 1990), a tolerable daily intake (TDI) of 10 pg/kg b.w. for TCDD was established. Since then new toxicological, epidemiological and mechanistic data have emerged, in particular with respect to neurodevelopmental, reproductive and endocrine effects. Therefore, WHO-ECEH and IPCS jointly organized a consultation on the Assessment of the health risk of dioxins: re-evaluation of the Tolerable Daily Intake (TDI). The consultation was held from 25-29 May 1998 at WHO Headquarters, Geneva, Switzerland, and was attended by 40 experts from Australia, Belgium, Canada, Denmark, Finland, Germany, Italy, Japan, The Netherlands, New Zealand, Spain, Sweden, United Kingdom, and USA and by staff from UNEP, IARC, IPCS and WHO-ECEH. Dr John Christian Larsen was elected chairman and Dr William Farland co-chair, Dr Mark Feeley and Professor Dieter Schrenk were elected co-rapporteurs, Drs F.X.Rolaf van Leeuwen and Maged Younes were joint scientific secretary. Financial support was provided by the German Ministry of the Environment, The Netherlands Ministry of Health, Well-being and Sports, and Health Canada.

As a basis for the discussion during the Consultation, working papers were prepared in advance of the meeting on: the health risks for infants, cancer and non-cancer endpoints in humans and animals, mechanistic aspects, toxicokinetics, modeling, exposure, the applicability of the TEQ concept, and risk assessment approaches for dioxins in various countries.

The meeting was opened by Dr R.van Leeuwen (WHO-ECEH) who welcomed the participants and presented the objective of this joint ECEH/IPCS meeting: the health risk assessment of dioxins considering both classical risk assessment methodologies and probabilistic risk assessment approaches, with a view to establishing a TDI for dioxins. He stressed the importance of a thorough, scientific evaluation of all available data and underlined the need for transparency in the derivation of a TDI.
Dr M. Mercier (Director IPCS) welcomed the participants to WHO Headquarters and expressed his appreciation for the close collaboration between WHO-ECEH and IPCS in this matter. He noted that the risk assessment of dioxins is a global effort rather than just a European endeavour, as could also be seen from the geographical

representation of the experts, and he expressed his confidence in the scientific expertise of the participants of the meeting to carry out the important task they had been given.

EXPOSURE

Background exposure

Human exposure to PCDDs, PCDFs, and PCBs may occur through background (environmental) exposure, and accidental and occupational contamination.
Over 90 percent of human background exposure is estimated to occur through the diet, with food from animal origin being the predominant source. PCDD and PCDF contamination of food is primarily caused by deposition of emissions from various sources (e.g. waste incineration, production of chemicals) on farmland and waterbodies followed by bioaccumulation up terrestrial and aquatic foodchains. Other sources may include contaminated feed for cattle, chicken and farmed fish, improper application of sewage sludge, flooding of pastures, waste effluents and certain types of food processing. The available information derived from numerous studies in industrialized countries indicates a daily intake of PCDDs and PCDFs in the order of 50-200 pg I-TEQ/person/day, or 1-3 pg I-TEQ/kg bw/day for a 60 kg *adult*. This results in average human background levels in the range of 10-30 pg I-TEQ/g lipid, equivalent to a body burden of 2-6 ng I-TEQ/kg body weight. If the dioxin-like PCBs (non-ortho and mono-ortho PCBs) are also considered, the daily TEQ intake can be a factor of 2-3 higher. Special consumption habits, particularly one low in animal fat or consumption of highly contaminated food stuffs may lead to lower or higher TEQ intake values, respectively. The intake of PCDDs/PCDFs and PCBs increases during childhood and stabilizes in adults of about 20 years of age. However, the intake on a per kilogram basis decreases in this period due to the increasing body weight. Despite differences in the absolute levels of PCDDs/PCDFs/PCBs, the congener profiles caused by background contamination are usually very similar. Recent studies from countries which started to implement measures to reduce dioxin emissions in the late 80s, such as The Netherlands, United Kingdom and Germany, clearly show decreasing PCDD/PCDF and PCB levels in food and consequently a significantly lower dietary intake of these compounds by almost a factor of 2 within the past 7 years.

Compared to adults, the daily intake of PCDDs/PCDFs and PCBs for *breast fed babies* is still 1-2 orders of magnitude higher on a per body weight basis. The latest WHO field study showed differences between the PCDD/PCDF and PCB contamination of breast milk, with higher mean levels in industrialized areas (10-35 pg I-TEQ/g milk fat) and lower mean levels in developing countries (< 10 pg I-TEQ/g milk fat). Within one country an individual variation of a factor of 5-10 was observed for most congeners, mainly due to age of the mother, number of breastfed babies, length of nursing period and consumption habits. There is now clear evidence of a decrease in PCDD/PCDF levels in human milk over time in almost every region for which suitable data exist. The WHO field study also showed that the highest rates of decrease have been in the areas with the highest initial concentrations. Latest results from Germany revealed a decrease of PCDD/PCDF levels in human milk of

approximately 65% between 1989 and 1997. These data support the substantial reduction in intake of PCDDs and PCDFs in the past few years.

Accidental exposure
Well-known examples of accidental exposure of the local population to PCDDs, PCDFS and PCBs include the incident at Seveso, and fires in PCB filled electrical equipment. In Seveso, the serum levels for 2,3,7,8-TCDD ranged up to 56000 pg/g lipid, with median levels of 450 pg/g lipid for Zone A and 126 pg/g lipid for Zone B. High exposure may also be caused by food items accidentally contaminated. Known examples are the contamination of edible oil, such as the Yusho (Japan) and Yu-Cheng (Taiwan) food poisoning. For a group of Yusho patients, average intake by ingestion of the Kanemi rice oil contaminated with PCBs, PCDFs and polychlorinated quarterphenyls (PCQs) was estimated at 154000 pg I-TEQ/kg bw/day, which is five orders of magnitude higher than the reported average background intake in several countries.

Occupational exposure
Industrial activities in which 2,3,7,8-TCDD and related compounds are unintentionally produced, such as waste incineration or production of certain pesticides or chemicals may also result in additional human exposure. While many industrial sources of 2,3,7,8-TCDD and related compounds have been identified and worker exposure has been reduced or eliminated historic median 2,3,7,8-TCDD levels in blood of highly exposed workers, estimated by extrapolation back to the time of last exposures, ranged from 140 to 2000 pg/g lipid. These estimates are 1-3 orders of magnitude higher than the blood levels measured in the general population. Body burdens caused by accidental or occupational exposure show congener patterns that are different from background exposure and are normally dominated by only a few congeners. This is because of direct exposure vs. indirect exposure through the food supply where bioaccumulation may modify congener patterns.

MECHANISM OF ACTION

A broad variety of data primarily on TCDD but also on other members of the class of dioxin-like compounds has shown the importance of the Ah (dioxin) receptor in mediating the biological effects of dioxin. These data have been collected in many experimental models in multiple species including humans. The precise chain of molecular events by which the ligand-activated receptor elicits these effects is not yet fully understood. However, alterations in key biochemical and cellular functions are expected to form the basis for dioxin toxicity. Pharmacological structure-activity and mouse genetic studies using Ah receptor-deficient animals and cells have demonstrated a key role for the receptor in mediating toxic effects of TCDD. For instance, a reduction or lack of acute toxicity in receptor-deficient mice has been documented. The activated receptor exerts two major types of functions: enhancement of transcription of a battery of genes containing responsive elements in their promoter regions, and immediate activation of tyrosine kinases. A number of genes encoding drug-metabolizing enzymes, such as cytochrome P4501A1, 1A2, 1B1, glutathione S-transferase, and UDP-glucuronosyltransferase are members of an Ah receptor target gene battery. Alteration of expression of other networks of genes may be directly or indirectly regulated by the Ah receptor. Activation of the receptor by a ligand can result in endocrine and paracrine disturbances and alterations in cell functions including growth and

differentiation. Some of these effects have been observed both in humans and animals, suggesting the existence of common mechanisms of action.

TOXICOKINETICS

The toxicokinetic determinants of dioxin and related chemicals depend on three major properties: lipophilicity, metabolism, and binding to CYP1A2 in the liver. Lipophilicity increases with more chlorination and controls absorption and tissue partitioning. Metabolism is the rate-limiting step for elimination. The persistent compounds are slowly metabolized and eliminated, and therefore bioaccumulate. Induction of CYP1A2, which is partially under the control of the aryl hydrocarbon receptor (AhR), leads to hepatic sequestration of TCDD. The structure/activity relationships for induction are different from that for binding to CYP1A2. Binding to this inducible hepatic protein results in non-linear dose dependent tissue distribution: as the dose increases, the relative concentration in extra-hepatic tissues decreases while that in liver increases. The induction of this protein occurs in both animals and people and results in a increase in the liver to fat ratio of these compounds. This effect has a minor impact on free TCDD and serum TCDD at the range of environmental exposure.

The basic determinants of pharmacokinetic behaviour are similar in animals and people. Several robust classical and physiologically based models have been used to describe the kinetic behaviour. They have contributed to the understanding that the apparent half-life is not absolute, but may vary with dose, body composition, age, and sex.

Given that these are persistent, bioacumulative compounds, what is the appropriate dose metric to use to equate risk across species? Free concentration in the target tissue would be the most appropriate measure. However, the body burden, which is highly correlated with tissue and serum concentration, integrates the differential half-lives between species. Much higher daily doses are required in rodents to achieve the same body burden, or tissue concentration, as a lower daily dose in people. Body burden is readily estimated in both people and rodents. Therefore, in order to compare risks between humans and animals, the body burden is the metric of choice. It is important to note that predictions of body burden based on lipid concentrations at high exposures may underestimate the total body burden and over- or underestimate specific tissue concentrations because of the hepatic sequestration. Use of PBPK models can readily allow for interconversion of body burden with tissue concentrations, as well as with daily dose. Less complicated models such as a steady state/ body burden models using first order kinetics will give approximately the same results at exposures in the environmental range.

There is a range of apparent half-lives for the various PCDDs, PCDFs, and dioxin-like PCBs. However, the TEQ is driven by a relatively small subset of these compounds. When background exposures are involved, an average half-life similar to that of TCDD may be used, but will underestimate daily exposure in short half-life chemicals and overestimate exposure for those with longer than average half-lives. However, if high levels of exposure are involved, such as in occupational settings, it is important to include the pharmacokinetic data on the individual chemicals.

Table 1. MOST SENSITIVE EFFECTS OF 2,3,7,8-TCDD IN ANIMALS

Effect	**Species**	**Exposure** (LOEL or LOAEL)	**Maternal body burden** (increment to background)*
Adverse effects			
Developmental effects - neurotoxicity (object learning)	Rhesus monkey	~160 pg/kg/d	42 ng/kg**
Reproductive toxicity - decreased sperm count - vaginal threads	rat	 64,000 pg/kg*** 200,000 pg/kg***	 28 ng/kg 73 ng/kg
Immunotoxicity	rat	100,000 pg/kg***	50 ng/kg
Immunological (viral sensitivity)	mouse	10,000 pg/kg***	10 ng/kg
Hormonal (endometriosis)	Rhesus monkey	~160 pg/kg/d	42 ng/kg**
Effects which may or may not lead to adverse effects			
Biochemical effects			
-CYP1A1	mouse rat	150 pg/kg/d 100 pg/kg/d	3 ng/kg 3 ng/kg
-CYP1A2	mouse	450 pg/kg/d	10 ng/kg
-EGFR	rat	100 pg/kg/d	3 ng/kg
-IL1beta	mouse	300 pg/kg/d	~10 ng/kg
Functional effects			
-oxidative stress	mouse	450 pg/kg/d	10 ng/kg
-lymphocyte subsets	marmoset monkey	~200 pg/kg/d	6-8 ng/kg

* Background body burden in rats and mice is about 4 ng/kg (TEQ)
** Body burden at the end of dosing period
*** Note: single dose

EFFECTS IN LABORATORY ANIMALS

Non-carcinogenic effects

A plethora of effects have been reported to occur in multiple animal studies following exposure to PCDDs, PCDFs and PCBs. The most extensive dataset on dose-response effects is available for 2,3,7,8-TCDD; less information is available for the other dioxin-like compounds. Therefore the focus of the evaluation of the animal data is on the effects of 2,3,7,8-TCDD.

Due to the multitude of different effects at various dose levels the most sensitive toxic and biochemical endpoints are presented in Table 1. In this table information on the lowest daily doses or body burdens resulting in the observed effects are included. The effects observed are each characterized either as an adverse (toxic) effect or as a biochemical and functional effect. The biochemical effects observed at the lowest body burdens, or tissue concentrations are early expressions of cascades of events induced by dioxin-like compounds that may or may not result in adverse effects in the animal or its progeny.

Among the most sensitive endpoints (on a body burden basis) are: endometriosis, developmental neurobehavioral (cognitive) effects, developmental reproductive (sperm counts, female urogenital malformations) effects, and immunotoxic effects, both adult and developmental . The most sensitive biochemical effects are CYP1A1/2 induction, EGF-receptor down-regulation and oxidative stress (Table 1).

The lowest doses giving rise to statistically significant effects in the most sensitive endpoints following exposure, have resulted in body burdens (e.g. 3 to 73 ng of TCDD/kg) in the exposed animals that overlap, at the lower end, the range of body burdens expressed as TEQ that are found in the general population in industrialized countries exposed to background levels of PCDDs,PCDFs and PCBs.

Carcinogenic effects

2,3,7,8-TCDD has been shown to be carcinogenic in several chronic studies at multiple sites in multiple species in both sexes. Short-term studies observed a lack of direct DNA-damaging effects including covalent binding to DNA by TCDD, which underscores that TCDD is not acting as an initiator of carcinogenesis. However, secondary mechanisms may be important in the observed carcinogenicity of TCDD and related dioxin-like compounds.

The lowest observed adverse effect of TCDD in the Kociba study was the development of hepatic adenomas in rats at an intake of 10 ng/kg bw/day and the no observed effect level was 1 ng/kg/day. At the no observed effect level, body burdens were 60 ng TCDD/kg bw. TCDD also causes thyroid tumours in male rats. This has been indicated to proceed through a mechanism which involves altered thyroid hormone metabolism, and consequent increases in feedback mechanisms (TSH) which results in a chronic proliferative stimulation of thyroid follicular cells.

Studies in the mouse skin support a lack of initiating activity and an ability to promote the growth of previously initiated lesions indicative of a promoting agent. Mouse skin tumour promotion indicates that the Ah receptor is involved in tumour promotion by TCDD. Extensive examination of liver tumour promotion in the female rat liver also

supports a non-genotoxic mechanism for the induction of liver neoplasms by TCDD. The ability of TCDD to enhance proliferation and inhibit apoptotic processes in focal hepatic lesions further supports an indirect mechanism of carcinogenicity.

Several PCDDs, PCDFs, non-ortho and mono-ortho PCBs have also been shown to be tumour promoters.

EFFECTS IN HUMANS

In the evaluation of the evidence of effects of PCDD, PCDFs and PCBs, only studies with serum or adipose tissue measurements were considered.

Human carcinogenicity data

The most informative studies for the evaluation of the carcinogenicity of 2,3,7,8-TCDD are four cohort studies of herbicide producers (one each in the United States and the Netherlands, two in Germany), and one cohort of residents in a contaminated area from Seveso, Italy. In addition, the multi-country cohort study from IARC includes three of the four high-exposure cohorts and other industrial cohorts, many of them not reported in separate publications, as well as some professional herbicide applicators.

In most epidemiological studies considered exposure was to mixtures of PCDDs including TCDD, as contaminants of phenoxy herbicides and chlorophenols. The cohorts examined in these epidemiological studies do not allow an evaluation of the risk associated with exposure to higher PCDDs separate from exposure to TCDD. These studies involve subjects with the highest recorded exposures to 2,3,7,8-TCDD. In these cohorts the blood lipid levels of 2,3,7,8-TCDD estimated to the last time of exposure were 2000 ng/kg (mean) (up to 32,000 ng/kg) in the US cohort, 1434 ng/kg geometric mean (range 301 –3683 ng/kg) among workers involved in the clean up of a TCP reactor accident in the Dutch cohort, 1008 ng/kg (geometric mean) in the group of workers with severe chloracne in the accident cohort in Germany, and up to 2252 ng/kg in the Boehringer cohort in Germany. These calculated blood 2,3,7,8-TCDD levels of workers at time of exposure were in the same range as the estimated blood levels in the Kociba two-year rat carcinogenicity study. Exposures in Seveso (median in zone A, 443 ng/kg; median in Zone B, 94 ng/kg) were, on average, lower than those of the industrial cohorts. The upper range of the high-exposed individuals was similar to that of the occupational cohorts (upper 75th percentile in Zone A, about 2000 ng/kg); there were 736 persons in Zone A.

Increased risks for all cancers combined were seen in the occupational cohort studies. The magnitude of the increase was generally low; it was higher in sub-cohorts considered to have the heaviest 2,3,7,8-TCDD exposure. Positive dose – response trends for all cancers combined were present in the largest and most heavily exposed German cohort, and in the smaller German cohort where an accident occurred with release of large amounts of 2,3,7,8-TCDD. Increased risks for all cancers combined were also seen in the longer-duration longer-latency sub-cohort of the United States study, and among workers with the heaviest exposure in the Dutch study. These positive trends with increased exposure tend to reinforce the overall positive association between all cancers combined and exposure. The large German cohort evaluated dose-response both for estimated exposure to TCDD and for PCDD/PCDFs using I-TEQ and identified a positive trend in both

analyses. In Seveso, all-cancer mortality did not differ significantly from that expected, in any of the contaminated zones, although excess risks were seen for specific cancers. Follow-up for the Seveso cohort was shorter than for the occupational cohorts. In most of these studies excess risks were observed for soft tissue sarcoma and also for lung cancer, non-Hodgkin lymphoma and digestive tract cancers. Statistically significant excess risks were observed in individual cohorts for a variety of other cancer sites including multiple myeloma, oral cavity, kidney cancer, leukaemia and breast cancer in women.

A single study in Seveso examined cancer in children 0-19 years of age. Excess risks were observed for ovarian and thyroid cancer and for some neoplasia of the haematopoietic tissue; these results were based on small numbers.

Two studies have evaluated cancer risk among subjects exposed to contaminated rice oil in Japan (Yusho) and Taiwan (Yucheng). The Japanese oil contained in the order of 1000 mg/kg PCBs and 5 mg/kg PCDFs. Estimates of intake are based on a study of 141 cases (Masuda, 1994). These patients consumed about 600 ml of oil over about one month, and ingested about 600 mg of PCBs and 3.5 mg of PCDFs total. Assuming a body weight of 60 kg, the daily dose was thus: 0.33 mg PCBs/kg/day and 0.002 mg PCDFs/kg/day. The Taiwanese oil contained about 100 mg/kg PCBs and 0.4 mg/kg PCDFs. Estimates are based a study of 99 cases. Patients consumed about 1 gram of PCBs and 3.8 mg of PCDFs over a period of about 10 months. Daily doses were approximately 0.06 mg PCBs/kg/day and 0.0002 mg PCDFs/kg/day. The contaminated rice oil contained a complex mixture of chlorinated ring compounds, including dioxin- and non-dioxin-like PCBs, PCQuaterphenyls, PCTerphenyls, as well as the PCDFs. There was an excess liver cancer risk in Japan (OR = 3.1) at 22 years of follow-up, and no excess risk in Taiwan (OR = 0.8) at 12 years.

In summary, the epidemiological evidence from the most highly 2,3,7,8-TCDD- exposed cohorts studied produces the strongest evidence of increased risks for all cancers combined, along with less strong evidence of increased risks for cancers of particular sites. The relative risk for all cancers combined in the most highly exposed and longer-latency sub-cohorts is 1.4. While this relative risk is not likely to be explained by confounding, this possibility cannot be excluded. It should be borne in mind that the general population is exposed to 2-3 orders of magnitude lower levels of TCDD, and 1-2 orders of magnitude lower levels of PCDD/PCDFs than those experienced, as an equivalent lifetime dose in the industrial populations examined or the population at Seveso.

Non-cancer effects in children

Two US birth cohorts with measured background exposure to PCBs have been followed since 1980, and 2 Dutch birth cohorts with measured background levels of PCBs, PCDDs and PCDFs have been followed since 1990. In Asia, some data are available on Japanese children exposed transplacentally to contaminated rice oil, and detailed follow-up is available on transplacentally exposed children in Taiwan. The estimated upper 10 percentile of total PCBs in breast milk lipid among mothers in the US cohorts was around 1.5 mg/kg; measurement of specific PCBs was limited by analytical methods available at the time of the studies. Mothers in these US cohorts were also exposed to other chlorinated pesticides and heavy metals. In the Dutch cohorts the mean TCDD I-TEQ concentration in human milk was 30.2 pg/g lipid (range 11.1 - 76.4 pg/g) and the estimated PCB concentration was 0.64 μg/g lipid.

Neurodevelopmental delays and neurobehavioral effects including neonatal hypotonia occurred in the three largest cohorts, two in the US and one in The Netherlands, although the age at which the effects occurred and the tests used to detect them were not the same. In the two US cohorts the observed neurobehavioral effects were limited to the infants with the highest decile of transplacental exposure, with some indication of a non-linear effect.
Thyroid hormone levels were evaluated in the two cohorts in The Netherlands with similar exposure to PCDDs/DFs and total PCBs. In utero exposure to total TEQs, as measured in mother's milk, may have influenced thyroid hormone status (TT4, TSH) in infants up to 3 months of age. In Japan and Taiwan effects on children exposed transplacentally to the contaminants in the rice oil included ectodermal defects, global persistent developmental delays, low birth-weight, mild persistent behaviour disorders, decrease in penile length at puberty, reduced height among girls at puberty and hearing loss. It should be noted however, that it is not clear to what extent dioxin-like and/or non-dioxin-like compounds are contributing to these effects when considering the complex mixtures that human individuals are exposed to. In all the studies of infants and children, effects were primarily associated with *in utero*, rather than lactational exposure. Breast fed infants in the Rotterdam/Groningen cohort were shown to have better neurobehavioural development compared to formula fed infants. Within the group of breast-fed infants, however, those with higher exposure within the cohort to total TEQs (> 50 pg/g milk fat) tended to have poorer neurobehavioral test results (Bayley PDI) compared to those with lower exposure (< 50 pg TEQs/g milk fat).

In children in Seveso who were highly exposed to TCDD, small, transient increases in hepatic enzymes, total lymphocyte counts and subsets, complement activity, and non-permanent chloracne were observed.Also an alteration of the sex ratio (excess female to male) was observed in children born to parents highly exposed to TCDD.

Non-cancer effects in adults
Several persistent, exposure-related effects occurred in two or more adult populations exposed to PCDDs, PCDFs and PCBs. These populations include the industrial cohorts previously described: US Air Force Ranch Hands (exposed to TCDD during spraying of Agent Orange, median serum TCDD levels back-extrapolated at time of exposure around 50 pg/g lipid), Centers for Disease Control Vietnam Experience Study (exposed to TCDD during a one year tour in Vietnam, mean serum TCDD levels at time of study in 1987, 4 pg/g lipid), and the Seveso, Yusho and Yucheng cohorts. The effects are elevated GGT in the NIOSH, Ranch Hands and Vietnam Experience cohorts (NIOSH: out-of-range GGT levels, OR=2.27, 95%CI, 1.17-4.39: Vietnam Experience Study: OR 1.3, 95%CI, 1.0-1.8: Ranch Hand mean GGT concentration in highest exposed group 33.3 pg/g lipid TCDD compared to referent group, $p<0.001$); statistically nonsignificant dose-related increases in triglyceride levels in the NIOSH cohort (and significant increases in Ranch Hands with serum TCDD concentrations above 15 pg/g lipd; significantly increased mean fasting plasma glucose levels among Ranch Hands with 2,3,7,8-TCDD concentrations $\geq$ 94 pg/g lipid (OR=1.5, 95%CI 1.2, 2.0), an increased prevalence of diabetes among workers in the NIOSH cohort with serum concentrations above 1500 pg/g lipid and mortality from diabetes among females in all zones of Seveso, particularly in zone B (Zone A, Obs=2, RR=1.8, 95%CI 0.4-7.3; Zone B, Obs=13, RR=1.9, 95%CI 1.1-3.2; Zone R, Obs=74, R.R. 1.2, 95%CI 1.0-1.6). Increased mortality from cardiovascular diseases occurred in

multiple industrial cohorts and in males of Zones A and R of the Seveso cohort. Positive dose-response trends were also observed for ischaemic heart disease in the heavily

Table 2. Estimated tissue concentrations in human populations exposed to dioxin and dioxin-like compounds

Study Population (date of exposure)	Exposure Circumstance	Primary Exposure	No. of Samples	Serum Level at Time of Study (pg/g lipid)	Estimated serum concentration at last exposure (pg/g lipid)	Estimated body burden at last exposure (ng/kg.bw)[1]
Yusho (1968)	Japan: Ingestion of contaminated rice oil	PCBs PCDFs PCQs PCTs		Adipose tissue wet weight within 1 year of end of exposure. 2.8 mg/kg PCBs, 5.7 mg 2,3,4,7,8 PeCDF, 1.7μg Hx total	NA	600 mg PCBs 3.5 mg PCDFs (60 kg bw)
Yucheng (1979)	Taiwan: Ingestion of contaminated rice oil	PCBs PCDFs PCQs PCTs		Lipid adjusted serum within 1 year of end of exposure 60 mg/kg total PCB (range 4-188) 0.14 μg/kg PCDF (range 0-0.27)	NA	Total ingested dose: 1000 mg PCBs 3.8 mg PCDFs
Seveso (1976)	TCP reactor release	TCDD	1976 Zone A: 296 Zone Bmax:80 Zone R: 48 1992 Zone A: 6 Zone B*: 52 Zone R: 52	1976 Zone A: ND-56,000 (median=450) Zone B: ND -1450 (median=126) Zone R: ND-100 (median= 50) 1992 Zone A: 61.5 (mean) 71.5 (median) Zone B: 16.8 (mean) 12.5 (median) Zone R: 5.3 (median) 5.5 (median)	 Zone A:333.8 388.7 Zone B: 111.4 77.6	 67 78 22 16

* randomly selected

Table 2. Estimated tissue concentrations in human populations exposed to dioxin and dioxin-like compounds (cont'd)

Industrial Cohorts						
NIOSH	TCP production	TCDD	253	ND-3300 median: 90 mean: 220	ND-32,000 median: 2,000	ND-6400 median: 400
Netherlands			48	geom. mean 22.9 (production) 87.2 (accident)	geom. Mean 286 (17-1160) 1434 (301-3683)	geom.mean 57 (3-232) 287 (60-737)
BASF			138	15.4 (geom) 84.1(median): 5 yrs after end of experiment	approx. 400	approx. 80
Boehringer-Ingelheim			48	48.9 (median): 11yrs after end of experiment	141 (3-2252)	28 (1-450)
U.S. Air Force Ranch Hand	Aerial spraying of Agent Orange in Vietnam	TCDD		ND-800 median:12.4	approx. 50	approx. 10
General population (Germany, 1996)	Background	PCDD PCDF	139	mean: 16.1 median: 15.2 Lowest: 7.3 95% 26.7	NA	range: 1.5-5 ng (I)TEQ/kg.bw

exposed German occupational cohort study, the Dutch occupational cohort and the IARC multicenter study. Among Yusho and Yucheng adults, chronic exposure-related effects included chloracne, conjunctivitis, and sebaceous cysts and inflammation, decreased nerve conduction velocity, fatigue and malaise, hyperpigmentation and hyperkeratosis, and increased mortality from non-malignant liver disease.

In summary, noncancer endpoints were evaluated among groups exposed to dioxins, dioxin-like and non-dioxin-like polychlorinated aromatic compounds in a variety of exposure scenarios, from background to extremely high exposures. Among children exposed *in utero* to background levels, effects include subtle developmental delays (U.S.and Dutch children) and subtle thyroid hormone alterations (Dutch infants to age 3 month). Multiple, persistent effects occurred among highly exposed children in Yusho and Yucheng who had transplacental exposure. Of the many effects evaluated in exposed adult study populations, many were transient effects disappearing after the end of exposure. A few conditions appear to be in excess among the exposed cohorts when compared to unexposed referent groups including alterations in lipid, fasting plasma glucose and GGT concentrations as well as mortality from cardiovascular disease. Both of the Asian cohorts showed excess death from non-malignant liver disease.

DOSE-EFFECT MODELLING

The key issues concerning modelling which were discussed included: the appropriateness of the data sets for the relevant endpoints, the kind of model used, the uncertainties in the model, and the transparency of the model with regard to the base assumptions. The model requires validation, e.g. with multiple data sets, before acceptance; this is often not done. In addition, use of raw data rather than summary data will substantially improve the models.

Human Cancer

The choice of data sets is determined to a large extent by the richness and completeness of the data. Therefore, in modelling human cancer, the most useable data sets are the industrial cohorts discussed by IARC in their 1997 monograph. In all these cohorts, exposure is back calculated from serum levels measured after the exposure had ceased. Average body burden over a lifetime was estimated assuming constant background levels of exposure before and after employment , and an assumption of continuous exposure to TCDD alone in the work place. The back calculation from the lipid adjusted serum levels observed after the end of the industrial exposure assumed a constant half-life of 7.1 years. A multiplicative linear hazard model was used to estimate a slope, using a maximum likelihood estimate. The ED01 to maintain the steady state body burden associated with a 1% excess risk over a lifetime results in a body burden of 3 -13 ng/kg, which is associated with a daily dose in the range of 2-7 pg/kg/day. If risk is related to peak exposure, rather than to continuous exposure, the estimate would be low. If the majority of exposure in the studied cohorts occurred within the earliest year instead of uniformly over the span of employment, the ED01 would increase by approximately a factor of three. It is important to note that the average exposure over time is not very different from these values (e.g., in the NIOSH cohort in the lower “exposure” group, those with less than one year of occupational exposure, resulted in an average lifetime body burden of approximately 10 ng/kg). However, the model does assume linearity within the range of the data, which is likely to provide a conservative position.

Experimental cancer studies
Two approaches to modelling were used: mechanistic and curve fitting. It is important to note that the mechanistic model had many assumptions and that other assumptions may be equally plausible and can lead to other models that adequately describe the data sets.

Mechanistic Model
Molecular, cellular, and promotion data was used to predict the incidence of liver tumours in the female Sprague Dawley rat observed in the Kociba study. The model assumed that dioxin exposure induced increased cellular proliferation and indirectly led to an increase in mutation rate due to induction of hepatic enzymes leading to oxidative stress. The hypothesis of no mutational effect was tested and could not be rejected for this model. Each part of the model was allowed to vary independently and was not constrained *a priori* to either a linear or nonlinear association. The ultimate best fit linear model lead to an excess 1% lifetime cancer risk associated with a steady state body burden of 2.6 ng/kg, resulting from a daily exposure of 150 pg/kg/day. Thus, while the ED01 on a daily dose base for rodents is much higher than that for humans, the steady state body burden for rodents is in the same range as that estimated for humans. This is due to the pharmacokinetic differences between the species.

Curve Fitting Model
The Armitage-Doll model was used to calculate a shape parameter to describe the results of multiple animal tumour studies in both rats and mice. The shape of the curve could be fitted by either linear or various non-linear power functions. 8 Out of 13 studies were best fitted by a linear model. However, the data may be described by a non-linear model. The ED01 based on a steady state body burden ranged from 10 ng/kg to 746 ng/kg, associated with daily doses of 1.3 ng/kg/day to 41.4 ng/kg/day. If these results are compared to the human cancer estimates, the body burdens again are similar, but the daily doses, as expected from pharmacokinetic considerations, are much higher in rats and mice. The animal estimates do not involve a large extrapolation to go from the observed data to a calculated ED01.

Non-cancer
No models have been evaluated for non-cancer effects in humans. The animal data sets that were modelled involved those with at least four dose groups and those in which a maximal response was at least approached, if not achieved. The Hill equation was used with non-linear least squares to fit the parameters and weighted for the observed variance and the shaping function was applied to assess linearity or non-linearity. For multiple dose studies, the average body burden at steady state was calculated as in the cancer studies. For the bolus studies, the body burden was assumed to be equivalent to the administered dose or that estimated by calculation at the time of response measurement based on first order elimination kinetics.

Modelling of 45 non-cancer studies in rodents demonstrated that 21 were best fitted by a near-linear model, while 24 demonstrated non-linearity. The biochemical endpoints were mainly linear; but most of the clearly adverse endpoints were non-linear. However, the decline in sperm count following prenatal exposure was linear. When the ED01 for the biochemical endpoints was compared with the observed LOEL, the ED01 was often higher than the measured response. This may reflect the sensitive measurements that can be made for biochemical responses. For the decrease

in sperm count, the ED01 was lower than the LOAEL. This may reflect measurement sensitivity, study design, and complexity of response. In some studies, estimation of the maximum response was problematic and the biological plausibility of the curve fits was unclear, underlying the need for mechanistic models for non-cancer endpoints.

The utility of the models is that they allow a common method of comparison, e.g. a 1% excess response. Use of this methodology allows comparison across responses. This benchmark methodology is less sensitive to the "ability to detect" a response based on the different study designs used to assess different endpoints. It is important to note that the ED01 for many of the non-cancer endpoints ranged for <1 to 100 ng/kg body burden. This is also true for cancer.

Regarding the importance of modelling to the human risk assessment for dioxin, the predictions of effects were compared with the actual data. At times, obvious discrepancies arise, leading to caution in the use of models. While recognizing that modelling is not suitable for human risk assessment of dioxins yet, it provides additional insights into the observational data, and adds to the transparency of the review.

APPLICABILITY OF TEF CONCEPT

The complex nature of polychlorinated dibenzo-p-dioxin (PCDD), dibenzofuran (PCDF), and biphenyl (PCB) mixtures complicates the risk evaluation for humans. For this purpose the concept of toxic equivalency factors (TEFs) has been developed and introduced to facilitate risk assessment and regulatory control of exposure to these mixtures. TEF values for individual congeners in combination with their chemical concentration can be used to calculate the total TCDD toxic equivalents concentration (TEQs) contributed by all dioxin-like congeners in the mixture using the following equation which assumes dose additivity:

$$TEQ = \sum (PCDD_i \times TEF_i) + \sum (PCDF_i \times TEF_i) + \sum (PCB_i \times TEF_i)$$

The majority of studies assessing the manner in which binary and complex mixtures of dioxin-like PCDD, PCDF and PCB congeners interact to cause toxicity have demonstrated that the interaction does not deviate significantly from dose additivity. This includes investigations conducted in various classes of vertebrates (fish, birds and mammals) and on environmental relevant mixtures. TEFs for dioxin-like compounds apply only to AhR-mediated responses. The criteria for including a compound in the TEF scheme for dioxin-like compounds are that the compound must:

- Show a structural relationship to the PCDDs and PCDFs
- Bind to the Ah receptor
- Elicit Ah receptor-mediated biochemical and toxic responses
- Be persistent and accumulate in the food chain

To reassess the TEFs for mammals a WHO expert group recently applied a tiered approach in which results of animal toxicity studies, especially those involving (sub)chronic exposure, were given significantly more weight than results of *in vitro* or biochemical studies. The results of this activity are summarized in Table 3.

Table 3. WHO TEFs for human risk assessment based on the conclusions of the World Health Organization meeting in Stockholm, Sweden, 15-18 June 1997 (Van den Berg et al., 1998).

Congener	TEF value	Congener	TEF value
Dibenzo-p-dioxins		***Non-ortho PCBs***	
2,3,7,8-TCDD	1	PCB 77	0.0001
1,2,3,7,8-PnCDD	1	PCB 81	0.0001
1,2,3,4,7,8-HxCDD	0.1	PCB 126	0.1
1,2,3,6,7,8-HxCDD	0.1	PCB 169	0.01
1,2,3,7,8,9-HxCDD	0.1		
1,2,3,4,6,7,8-HpCDD	0.01	***Mono-ortho PCBs***	
OCDD	0.0001	PCB 105	0.0001
		PCB 114	0.0005
Dibenzofurans		PCB 118	0.0001
2,3,7,8-TCDF	0.1	PCB 123	0.0001
1,2,3,7,8-PnCDF	0.05	PCB 156	0.0005
2,3,4,7,8-PnCDF	0.5	PCB 157	0.0005
1,2,3,4,7,8-HxCDF	0.1	PCB 167	0.00001
1,2,3,6,7,8-HxCDF	0.1	PCB 189	0.0001
1,2,3,7,8,9-HxCDF	0.1		
2,3,4,6,7,8-HxCDF	0.1		
1,2,3,4,6,7,8-HpCDF	0.01		
1,2,3,4,7,8,9-HpCDF	0.01		
OCDF	0.0001		

Van den Berg, M., Birnbaum, L., Bosveld, B.T.C., Brunström, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., van Leeuwen, F.X.R., Liem, A.K.D.,Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt,D., Tysklind, M., Younes, M., Waern, F.,Zacharewski, T. Toxic Equivalency Factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environmental Health Perspective, 106 (12), 775-792, 1998

While additivity predominates in the majority of experimental studies, non-additive interactions of PCDD, PCDF and PCB mixtures have been reported at greater than environmental levels of exposure. These non-additive effects are considered to be due to multiple mechanisms of action of individual congeners and/or to pharmacokinetic interactions. For the *mono- ortho* PCBs especially, certain endpoints such as carcinogenicity, porphyrin accumulation, alterations in circulating thyroid hormone concentrations and neurotoxicity could arise by both Ah receptor-mediated and non-Ah receptor-mediated mechanisms.

In addition, non-Ah receptor-mediated mechanisms of action of the *mono-ortho* PCBs may be shared by certain di-, tri-, and tetra-chloro *ortho*-substituted PCBs. This increases uncertainty in the use of TEFs for *mono-ortho* PCBs. While recognizing that these and other uncertainties exist in the use of the TEF concept for human risk assessment, pragmatically it remains the most feasible approach. Use of TCDD alone as the only measure of exposure to dioxin-like PCDDs, PCDFs and PCBs severely underestimates the risk to humans from exposure to these classes of compounds. Thus, the TEF approach is recommended for expressing the daily intake in humans of PCDDs, PCDFs, *non-ortho* PCBs and *mono-ortho* PCBs in units of TCDD equivalents (TEQs) for comparison to the tolerable daily intake (TDI) of TCDD.

EVALUATION AND CONCLUSIONS

Exposure

Substantial information on the concentrations of PCDDs/PCDFs and limited information on dioxin-like PCBs in environmental samples, foods, human tissues, as well as breast milk are available for a number of mainly industrialized countries. The information indicates that the concentrations of these compounds have decreased during the last 10 years, mainly due to enforced regulations that have limited their dispersal to the environment and hence the food chains.

The available information derived from food surveys in numerous industrialized countries indicates a daily intake of PCDDs and PCDFs in the order of 50-200 pg I-TEQ/person/day, or 1-3 pg I-TEQ/kg bw/day for a 60 kg adult. This intake results in average human tissue levels in the range of 10-30 pg I-TEQ/g lipid, equivalent to a body burden of 2-6 ng I-TEQ/kg body weight. If the dioxin-like PCBs (non-ortho and mono-ortho PCBs) are also considered, the daily TEQ intake may be greater by a factor of 3-fold.

Based on results from the latest WHO field study on human breast milk contaminant concentrations, average PCDD/PCDF levels, expressed on an I-TEQ basis, ranged from less than 10 pg/g milk fat in developing countries to 10-35 pg/g milk fat in industrialized countries. When dioxin-like PCBs are included, the total TEQ concentration increases in the order of 2-fold. For example, in a large sample of Dutch breast milk samples collected in 1990-91, the mean concentration of PCDD/PCDF TEQs was 34.4 pg/g milk fat; when dioxin-like PCBs were included in the calculation, the total TEQ value increased to 72.3 pg/g milk fat. The average daily intake of a breast-fed infant, on a body weight basis, therefore may be almost 1-2 orders of magnitude greater than that of an adult. It should be noted that the majority of industrialized countries have recorded decreases of up to 50% in the concentration of PCDDs/PCDFs and total PCBs in breast milk within the past 10 years.

When TEQ calculations (based on 1997 WHO TEFs) for exposure and body burden are considered on an individual congener basis in background populations TCDD generally accounts for only 10-20 % of the PCDD/PCDF-TEQs. When dioxin-like PCBs are also included TCDD often contributes less than 5 % to the total TEQ.

The consultation recommended that the new TEFs for PCDD/PCDF and dioxin-like

PCB derived by WHO in 1997 (see Applicability of the TEF concept) should be used for future calculations of TEQs. This will result in an approximate 10% increase in TEQ calculations, compared to using I-TEFs and the initial 1994 WHO TEFs for PCBs.

Toxicokinetics

The key determinants in the kinetics and the half-lives of these compounds are amount of fat stores in the body, binding to CYP 1A2 in the liver, and rate of metabolism and excretion. The dose also plays a significant role and it was found that humans also sequester these compounds in the liver at higher doses, as do experimental animals. However, because of species variation in the above mentioned determinants, rodents require appreciably greater doses (100-200-fold) to reach the same equivalent body burdens as has been determined in humans exposed only to background concentrations to dioxin and related compounds. From a pharmacokinetic point of view, estimates of body burden are considered the most appropriate dosimetric parameter for interspecies comparison.

The existence of a relationship between average daily intake and resulting tissue levels in humans is supported by data from Germany which showed that decreases in average daily dioxin TEQ intake over the course of 7 years (1989-1996) were associated with similar declines in human milk and blood concentrations. Due to the relatively long half-lives in humans of dioxins and related compounds, steady-state body burden estimates usually reflect a stable condition in which brief intake above background will not result in significant changes to the body burden.

Health effects

The consultation noted that PCDDs, PCDFs, and the dioxin-like PCBs (non-ortho and mono-ortho substituted PCBs) exert a number of biochemical and toxicological effects mediated through the Ah receptor. Ah receptor binding affinity and responses directly dependent on Ah receptor activation suggest that humans may be less susceptible to TCDD than “responsive” rodent strains, whereas other biochemical or cellular effects are suggestive of a comparable susceptibililty. The broad range of Ah receptor binding affinities seen in human placenta samples suggests considerable variability of this parameter may exist within the general population.

A number of biochemical effects (CYP1A1/2 induction, EGFR down regulation, etc.) have been observed in experimental animals at body burdens comparable to those of the general human population. These effects may or may not have implications for the toxicity of TCDD.

In the course of evaluating the adverse effects of dioxins at low doses, the usefulness of toxicokinetic and dose-effects modelling to calculate a “benchmark” (ED01) for comparison in the assessment was explored. It was noted that the outcome of using such models would strongly depend on the assumptions used and there are still a number of uncertainties in the interpretation of the results. Therefore, more traditional approaches using simple body burden calculations and empirical observations (LOAELs and NOAELs) have been used in this evaluation.

As discussed earlier, a wide variety of effects has been observed in studies of TCDD, and to a more limited extent of other PCDDs, PCDFs and dioxin-like PCBs, in animals and also in studies of complex mixtures of these compounds in human populations. For

the purposes of a risk assessment of human exposure to dioxin-like compounds the consultation focused on effects seen at low doses. Table 1, Animal End-points non-cancer effects, presents a range of reported animal LOAELs which are considered adverse and which occur at body burdens in the range of 10-73 ng/kg. This suite of effects represents critical studies for the assessment of low dose effects of PCDDs/PCDFs.

Among these are developmental and reproductive effects in rats and monkeys. Responses are presented along with information on the increment to background body burdens in the experimental animals. These body burdens can readily be transformed into estimated daily human intakes that on a chronic basis would be expected to lead to similar body burdens in humans. Under steady state conditions, it is possible to estimate intakes as:

$$\text{Intake (ng/kg/day)} = \text{Body Burden (ng/kg)} * (\ln(2)/\text{half-life})/f$$

where f is the fraction of dose absorbed and is assumed to be 50% for absorption from food for humans, and an estimated half-life for TCDD of 7.5 years assumed. The results of such calculations appear in Table 4. Considering the very large differences in the half lives of dioxin-like compounds in various species, it is best to compare across species using this measure. It should be noted that the estimated human daily intakes are related to the body burdens in animals where adverse effects have been reported.

The consultation also considered a study of enhanced viral sensitivity in mice following acute exposure to TCDD but did not consider it appropriate for inclusion in the range of LOAELs as the lack of a dose-response relationship implies that there may be an unknown mechanism of action. In addition, children from Seveso with chloracne, who had been exposed acutely to high doses of TCDD, exhibited only minor transient alterations in various non-specific immune system parameters (see later). Similar analyses of sensitive responses in chronic animal cancer studies allow estimation of human daily intake values of about 150 pg/kg/day for the LOAEL (10 ng/kg/day) of the Kociba rat study corresponding to a body burden of 294 ng TCDD/kg, respectively. In addition to the adverse effects reported, numerous biochemical changes have been noted in experimental animals at body burdens within the range of 3-10 ng/kg. Several of these are also shown in Table 1. While these effects are observed at the lowest body burdens, they are considered to be early markers of events induced by dioxin-like compounds in animals and in humans and may or may not result in adverse effects.

In humans, maternal ingestion of high levels of a complex mixture of congeners from heat degraded PCBs (PCBs, PCDFs, PCQs) resulted in a variety of persistent severe adverse developmental and neurological effects in the infants. Maternal body burdens at the time of exposure were estimated to be 2-3 µg TCDD TEQs/kg. Non-cancer effects observed in mainly adult male workers occupationally exposed to high levels of TCDD and, to a lesser extent, higher chlorinated PCDDs included changes in serum lipids, elevated serum GGT, increased incidence of cardiovascular disease and diabetes. These effects were associated with mean body burdens at the time of last exposure ranging from 28-400 ng/kg.

TABLE 4. ANIMAL BODY BURDENS TCDD AND RELATED HUMAN ESTIMATED DAILY INTAKES (EDI)

STUDY	RESPONSE (LOAELs)	MATERNAL BODY BURDEN* (ng/kg bw)	RELATED HUMAN EDI (pg/kg bw/day)
Gray et al., 1997a	**RATS: decreased sperm count in offspring**	**28**	**14**
Gehrs et al., 1997b; Gehrs & Smailowicz 1998	**Immune suppression in offspring**	**50**	**25**
Gray et al., 1997b	**Increased genital malformations in offspring**	**73**	**37**
Schantz and Bowman, 1989	**MONKEYS: Neurobehavioural (object learning) effects in offspring**	**42**	**21**
Rier et al., 1993	**Endometriosis**	**42**	**21**

- **increment over background**

- **Gehrs, B.C., Riddle, M.M., Williams, W.C. and Smialowicz, R.J. Alterations in the developing immune system of the F344 rat after perinatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. II. Effects on the pup and the adult. Toxicology 122:229-240, 1997b.**
- **Gehrs, B.C. and Smailowicz, R.J. Persistent suppression of delayed-type hypersensitivity (DTH) in rats perinatally exposed to TCDD. Toxicologist 42:1501, 1998.**
- **Gray, L.E., Ostby, J.S. and Kelce, W.R. A dose-response analysis of the reproductive effects of a single gestational dose of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in male Long Evans hooded rat offspring. Toxicol. Appl. Pharmacol. 146:11-20, 1997a.**
- **Gray, L.E., Wolf, C., Mann, P. and Ostby, J.S. In utero exposure to low doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) alters reproductive development of female Long Evans hooded rat offspring. Toxicol. Appl. Pharmacol. 146:237-244, 1997b.**
- **Rier, S.E., Martin, D.C., Bowman, R.E., Dmowski, W.P. and Becker, J.L. Endometriosis in Rhesus Monkeys (*macaca mulatta*) following chronic exposure to 2,3,7,8,-tetrachlorodibenzo-p-dioxin. Fundam. Appl. Toxicol. 21:433-441, 1993.**
- **Schantz, S. and Bowman, R.E. Learning in monkeys exposed perinatally to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Neurotoxicol. Teratol. 11:13-19, 1989.**

In Seveso residents (children and adults) acutely exposed to high levels of TCDD alone resulting in median serum lipid TCDD concentrations of 450 ng/kg in individuals in the zone of highest exposure (zone A), a variety of transient effects were seen including chloracne, increases in a serum enzyme activity (GGT) and alterations in lymphocyte counts. Studies on children from zone A did not reveal effects on immune competence. Mortality studies have indicated an excess of deaths due to cardiovascular diseases in

males from zone A while an alteration in the sex ratio (excess females) of infants born to parents who both resided in zone A has also been reported.

As noted previously, back calculated blood concentrations of 2,3,7,8-TCDD determined in occupational cohorts which provide limited evidence of a human cancer response associated with dioxin exposure overlap with the blood concentrations determined in rats of the highest dose group (100 ng/kg/day) of the Kociba study. These and other data suggest that humans might be as sensitive as other animals to the adverse effects of dioxin and related compounds although data to evaluate comparable endpoints are frequently lacking.

The consultation recognized that the epidemiological evidence for the most highly TCDD-exposed cohorts studied produces the strongest evidence of increased risks for all cancers combined, along with less strong evidence of increased risks for cancers of particular sites. The relative risk for all cancers combined in the most highly exposed and longer latency sub-cohorts was 1.4. While the relative risk is not likely to be explained by confounding, this possibility cannot be excluded.

In the industrial populations or the population of Seveso in which cancer statistics were examined, the exposure to TCDD was higher by 2-3 orders of magnitude (to PCDD/DFs by 1-2 orders of magnitude) than that in the general population. The median body burdens associated with these exposures were 20 - 100 ng/kg.

The interpretation of the results from cohort studies concerning the effects on birth weight, maternal and new-born circulating thyroid hormones, and on the infant's developing nervous system is complicated by the simultaneous exposure to non-dioxin like PCBs (and maybe other compounds) that also might have played a significant role in eliciting these effects. These effects were observed at TEQ body burdens only slightly higher than that of the average general population, and thus point to the need for continuing efforts to reduce human exposure to these compounds, by controlling their input to the environment.

Derivation of TDI

Estimation of a TDI for dioxin and related compounds would require that either a reliable No-Adverse-Effect-Level (NOAEL) or a reliable LOAEL be identified for the most sensitive (and relevant) adverse response, that can serve as a surrogate for all other adverse responses that might be expected from exposure to these compounds. The LOAELs for the most sensitive adverse responses reported in experimental animals (Table 4) were associated with body burdens from which a range of estimated long-term human daily intakes of 14-37 pg/kg/ day was calculated. The consultation noted that the lower and upper end of this estimated range was related to effects following acute gavage (bolus) exposure to rats, but that this range also included effects seen after dietary exposure of monkeys for a prolonged period of time (4 years), the latter more resembling the conditions of human intake of these compounds. In view of the uncertainties in establishing a single, most appropriate LOAEL for derivation of a TDI, the consultation concluded that the range of estimated human daily intakes of 14 - 37 pg/kg/day provided a reasonable basis for the evaluation of the health risk of dioxin-like compounds. In order to arrive at a TDI based on TEQs, the use of uncertainty factors also had to be addressed in order to allow: a) the use of a range of LOAELs instead of a NOAEL, b) the possible differences between humans and experimental animals in

susceptibility to these compounds, c) the potential differences in susceptibilities within the human population, and d) differences in half-lives of elimination for the compounds of a complex TEQ mixture. Since body burdens have been used to scale doses across species, the use of an uncertainty factor to account for species differences in toxicokinetics is not required. With regards to the potential differences in susceptibility to the effects of these compounds, it is mentioned before that for some endpoints humans might be as sensitive as experimental animals to the adverse health effects of dioxin and related compounds. This implies that only a small uncertainty factor needs to be employed for differences in susceptibility. As the LOAELs presented in Table 4 were considered to be within a factor of 2-3 to the NOAELs, and the differences in half-lives between the dioxins and dioxin-like PCBs were also small (and partly accounted for in the establishment of the TEF values), the consultation was of the opinion that a composite uncertainty factor of 10 would be adequate.

By applying an uncertainty factor of 10 to the range of LOAELs of 14-37 pg TCDD/kg bw/day a TDI, expressed as a range, of 1 - 4 TEQ pg/kg bw (rounded figures) was established for dioxins and dioxin-like compounds.

The consultation emphasized, that the TDI represents a tolerable daily intake for life-time exposure and that occasional short-term excursions above the TDI would have no health consequences provided that the averaged intake over long periods is not exceeded. In addition, it recognized that certain subtle effects may be occurring in some sections of the general populations of industrialized countries at current intake levels (2-6 TEQ pg/kg bw/day) and body burdens (4-12 TEQ ng/kg bw), but found it tolerable on a provisional basis as these reported subtle effects were not considered overtly adverse and there were questions as to the contribution of non-dioxin-like compounds to the observed effects. The consultation therefore stressed that the upper range of the TDI of 4 pg TEQ/kg bw should be considered a maximal tolerable intake on a provisional basis and that the ultimate goal is to reduce human intake levels below 1 pg TEQ/kg bw/day.

The consultation therefore recommended that every effort should be made to limit environmental releases of dioxin and related compounds to the extent feasible in order to reduce their presence in the food chains, thereby resulting in continued reductions in human body burdens. In addition, immediate efforts should be made to specifically target exposure reductions towards more highly exposed sub-populations.

Breastfeeding

Breast-fed infants are exposed to higher intakes of these compounds on a body weight basis, although for a small proportion of their lifespan. However, the consultation noted that in studies of infants, breast feeding was associated with beneficial effects, in spite of the contaminants present. The subtle effects noted in the studies were found to be associated with transplacental, rather than lactational, exposure. The consultation therefore reiterated conclusions of previous WHO meetings on the health significance of contamination of breast milk with dioxin-like compounds; namely that the current evidence does not support an alteration of WHO recommendations which promote and support breast feeding. Based on new clinical data which supports the biological plausibility of certain experimental observations, continued and enhanced effort should be directed towards identifying and controlling sources of environmental input of these substances.

The consultation noted that within the last 10 years there is clear evidence of a decrease in dioxin levels in human milk in almost every region for which suitable data exists. This is most probably attributable to enhanced identification and control of environmental input sources. A future consultation in approximately 5 years should evaluate progress towards these goals.