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Risk assessment of non-developmental health effects of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans and dioxin-like polychlorinated biphenyls in food

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Contents

Abbreviations

2,4,5-T	2,4,5-tricholophenoxy acetic acid
AF	Assessment Factor
AGD	Anogentital distance
AhR	Aryl hydrocarbon Receptor
ARNT	AhR nuclear translocator
BB	Body Burden
BMD05	Bench Mark Dose at 5 percent Effect Level
BMDL05	Lower confidence bound on BMD05
BMI	Body Mass Index
bw	Body weight
CB	Chlorinated biphenyl
CEU	Council of the European Union
COT	Committee of Toxicology
CYP	Cytochrome P450
DDT	Dichlorodiphenyltrichloroethane
dl-PCB	Dioxin-like PCB
DTH	Delayed-type hypersensitivity
ECEH	European Centre for Environment and Health
EGFR	Epidermal Growth Factor Receptor
EHDI	Estimated Human Daily Intake
EPA	Environmental Protection Agency
ER	Estrogen Receptor
EROD	Ethoxyresorufine-*O*-deethylase
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FSA	Food Safety Agency
GGT	Gamma glutamyl transferase
GST	Glutation S Transferase
H2N2	Influenza virus type A/Taiwan/1/64
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HIF	Hypoxia Inducible Factor
IARC	International Agency for Research on Cancer
ICRP	International Commission on Radiological Protection
ICPS	International Programme on Chemical Safety
Ig	Immunoglobulin
IL	Interleukine
ip	Intraperitoneal
JECFA	Joint FAO/WHO Expert Meeting on Food Additives
NF	Nuclear Factor
NFA	National Food Administration
NIOSH	National Institute for Occupational Safety and Health

NOAEL/LOAEL	No/Lowest Observed Adverse Effect Level
NOEL/LOEL	No/Lowest Observed Effect Level
NTP	National Toxicology Programme
p,p'-DDE	*para, para´*-Dichlorodiphenyldichloroethylene
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-*p*-dioxin
PCDF	Polychlorinated dibenzofuran
PFC	Plaque Forming Cell Response
POP	Persistent Organic Pollutant
PROD	Pentoxyresorufine-*O*-deethylase
sc	Subcutane
SCF	Scientific Committee for Food
SMR	Standardized mortality ratio
SRBC	Sheep Red Blood Cells
STS	Soft-tissue sarcoma
T½	Half-life
T3	Triiodothyronine
T4	Thyroxine
TT4	Total thyroxine
TBG	Thyroid-hormone-binding-globuline
TCDD	2,3,7,8-tetrachloro dibenzo-*p*-dioxin
TDI/TWI/TMI	Tolerable Daily/Weekly/Monthly Intake
TEF	Toxic Equivalence factor
TEQ	Toxicity equivalence
TGF	Transforming Growth Factor
TSH	Thyroid Stimulating Hormone
TTP	Time to pregnancy
t-TWI	Temporary TWI
UDPGT	UDP-glucuronosyl transferases
WHO	World Health Organization

Preface

The Swedish National Food Administration (NFA) and the Institute of Environmental Medicine at Karolinska Institutet have performed a risk assessment of non-developmental exposure to polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs), in the following text called dioxins, as a scientific base for revision of the dietary advice regarding consumption of dioxin-contaminated fish in Sweden. The tolerable weekly/monthly intakes (TWI/TMI) established by the European Union (EU) [10, 16] and the World Health Organisation (WHO) [5] are based on toxicological studies of *in utero* exposure of rodents to the most toxic dioxin 2,3,7,8-tetrachloro-p-dibenzodioxin (TCDD). These TWI/TMI are consequently only relevant for girls and women of child-bearing age who anticipate becoming pregnant at some point. The TDI/TWI does not give information about tolerable exposure levels for boys, men and women over child-bearing age.

The aim of the risk assessment is to identify the critical health effects on humans exposed to dioxins during childhood and adulthood, and, if possible, to obtain a tolerable exposure level for these stages in life. In this process, uncertainties in the scientific data have been weighed into different scenarios of extrapolation from animal risks to human risks. Moreover, human data have been used as far as possible in the assessment of risks at background levels of exposure.

The risk assessment is one of several scientific inputs to the process of revision of fish consumption advisories performed by the Swedish NFA, and will be used by the risk managers during the development of the new advisory. In this process the weighing of risks connected to fish consumption against the benefits of fish consumption is important, and we believe that this risk assessment will give a valuable contribution to this analysis.

The risk assessment is based on the earlier risk assessments performed by the EU [10, 16] and WHO [5]. The critical studies regarding risks due to exposure during childhood (not including breast milk exposure), adolescence and adulthood were extracted from these assessments and from the literature published after these assessments were performed.

We focus on toxic effects due to exposure after birth (not including breast milk exposure), both in animals and humans. The report does not include in-depth information on chemistry and analytical methods, sources, environmental levels,

sources of human exposure, and human exposure. Information about these topics can be obtained from the earlier risk assessments of *in utero* exposure [5,10,16].

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Sammanfattning

Livsmedelsverket (SLV) och Institutet för miljömedicin (IMM) har slutfört en riskvärdering av barns och vuxnas exponering för polyklorerade dibenzo-*p*-dioxiner (PCDD), polyklorerade dibenzofuraner (PCDF) och dioxin-lika polyklorerade bifenyler (PCB), nedan kallade dioxiner. Riskvärderingen är ett av underlagen för den revision av Livsmedelsverkets konsumtionsråd gällande dioxinförorenad fet fisk som pågår under år 2006 och 2007. Det tolerabla intag av dioxiner, som EU:s och WHO:s expertgrupper föreslagit, baseras på toxikologiska studier av djur som exponerats under fosterstadiet. Detta tolerabla intag anger den långsiktiga intagsnivå som flickor och unga kvinnor inte bör överskrida för att kroppsbelastningen av dioxiner ska ligga på en säker nivå under graviditeten. Vår nya riskvärdering av dioxinexponering under barndom och vuxenliv riktar sig mot den del av befolkningen som inte kommer att genomgå en graviditet. Riskvärderingen är ett vetenskapligt bidrag till den hälsobaserade risk-nytta-analys som genomförs inom ramen för revisionen av konsumtionsråden.

Riskvärderingen baseras framförallt på toxikologiska studier av djur som exponerats för den mest giftiga dioxinen 2,3,7,8-tetraklorodibenzo-*p*-dioxin (TCDD). I tidigare riskvärderingar av TCDD har exponeringen uppskattats som halt av TCDD per kilo kroppsvikt. I SLV:s och IMM:s riskvärdering har vi utgått från halten i fettvävnad vid exponeringsuppskattningen, eftersom det antas att dioxiner vid låga exponeringsnivåer är jämt fördelade i fettet i kroppens olika vävnader och organ. Halten i fett ger en mer rättvisande bild av den exponering som målorganen får, än halten per kilo kroppsvikt. Genom att i riskvärderingen av människans exponering använda de TCDD-halter i fett som uppnåtts i djurförsöken, tar man hänsyn till att det finns skillnader i toxikokinetik mellan djur och människa. I beräkningar av TCDD-halt i kroppen antogs en fettmängd på 10 % i råtta och mus och 20 % hos människa. Dessutom användes en absorptionsfaktor för TCDD på 80 % istället för 50 %, som tidigare har använts i de internationella riskvärderingarna. Den högre absorptionen och de olika fettmängderna hos djur och människor stöds av publicerade data.

Djurstudier av TCDD-exponering har klart visat att fosterstadiet är den mest känsliga perioden, och att reproduktionssystemet är det mest känsliga målorgansystemet vid fosterexponering. Vid exponering av unga och vuxna djur tycks utveckling av cancer vara den mest känsliga negativa hälsoeffekten. Efter att råttor av båda könen exponerats under 2 år (livstidsexponering) uppskattades den högsta TCDD-halten i kroppen som inte orsakade cancerutveckling till 540-2000 ng/kg fett (No Observed Adverse Effect Level, NOAEL). TCDD är inte

genotoxiskt och det kan därför förväntas att det inte föreligger någon cancerrisk vid exponeringar under en viss tröskelnivå av exponering.

Benchmark-modellering användes för att uppskatta den exponeringsnivå som orsakar en ökning av cancerincidensen på 5 % i förhållande till bakgrundsincidensen i djurförsök (Benchmark dose, BMD). I modelleringen beräknades den lägre 95 % konfidensgränsen på BMD (BMDL). BMDL utnyttjades sedan i riskvärderingen. Från cancerstudierna i råtta uppskattades BMDL05 till 1500 ng TCDD/kg fett vilket motsvarade ett genomsnittligt dagligt intag av TCDD på 95 pg/kg kroppsvikt hos människa.

Eftersom den toxikologiska värderingen baseras på djurstudier, krävs en extrapolering av resultaten till en säker exponeringsnivå hos människa. Normalt sett används så kallade (o)säkerhetsfaktorer för att kompensera för eventuella skillnader i kinetik och toxikodynamik (känslighet) mellan djur och människor, samt mellan olika människor. Vi presenterar tre olika scenarier för användandet av säkerhetsfaktorer. I det första scenariot (scenario 1) utnyttjades den modell som använts av EU:s och WHO:s expertgrupper. De gjorde bedömningen att människan inte är känsligare för dioxiner än vad djur är och använde därför inga säkerhetsfaktorer för artskillnader eller individuella skillnader mellan olika människor gällande toxikodynamik. I scenario 1 använde vi alltså endast den säkerhetsfaktor (3,2 x) för skillnader i toxikokinetik mellan olika människor som EU:s och WHO:s expertgrupper använt, med anledning av att halveringstiden för TCDD i kroppen varierar mellan olika individer. Scenario 1 resulterade i en tolerabel fetthalt av TCDD hos människa på 500 ng/kg fett, vilket motsvarar ett dagligt intag på 30 pg/kg kroppsvikt/dag.

Vi bedömmer dock att det krävs ytterligare en säkerhetsfaktor för att ta hänsyn till möjliga skillnader i toxikodynamik i den mänskliga populationen (3,2 x). Både mekanistiska och epidemiologiska studier har antytt att det finns en stor variation i känslighet inom den mänskliga populationen. I epidemiologiska studier och de experimentella djurstudierna går det ofta inte att urskilja om känslighetsskillnaderna beror på variation i kinetik eller dynamik. Extrapoleringen i scenario 2, inkluderande faktorer både för skillnader i kinetik och dynamik bland människor, resulterade i en tolerabel fetthalt på 150 ng TCDD/kg kroppsvikt, motsvarande ett dagligt intag på 10 pg/kg kroppsvikt och dag.

I scenario 3 användes ytterligare en säkerhetsfaktor (5x) eftersom cancer är en allvarlig och ofta livshotande effekt. En extra säkerhetsmarginal mot BMDL05 motiveras också med att BMDL05 är en exponeringsnivå som innebär en signifikant ökning av cancerincidensen hos djur med 5 %, vilket kan anses vara oacceptabelt ur hälsomässig synvinkel. En faktor på 50 gånger från fetthalten av TCDD vid BMDL05 ger en tolerabel fetthalt på 30 ng TCDD/kg fett, motsvarande ett dagligt intag på 2 pg/kg kroppsvikt.

Riskvärderingen av TCDD kan även användas för andra PCDD/DF och dioxin-lika PCB om WHO:s system för toxiska ekvivalent faktorer (TEF) för dessa ämnen används vid beräkning av totalexponering för toxicitetsekvivalenter (TEQ).

Den nuvarande kunskapen om cancerrisker vid dioxinexponering bland människor visar att cancerrisken för människa sannolikt är mycket liten eller obefintlig vid långvarig exponering för nivåer motsvarande de som beräknats fram i scenario 2 (10 pg TEQ/kg kroppsvikt/dag) och 3 (2 pg TEQ/kg kroppsvikt och dag). Utgående från konsumtionsdata från den senaste populationsbaserade kost-undersökningen för vuxna i Sverige, Riksmaten 1997-98, har det långsiktiga medianintaget av TEQ för kvinnor och män i åldern 17-75 år uppskattats till 1,1 pg TEQ/kg kroppsvikt/dag. Denna intagsnivå ligger under det mest konservativa TDI:t på 2 pg TEQ/kg/dag. Även de högsta intagen (95:e percentilen, 2,9 pg TEQ/kg/dag) ligger endast marginellt över det mest konservativa TDI:t.

Intagsberäkningar baserade på kostundersökningen Riksmaten - barn 2003 har visat att medianintagen bland barn i åldern 4-12 år (1,2-2,3 pg TEQ/kg kropps-vikt/dag) ligger under eller i nivå med det mest konservativa TDI:t. Även intagen vid den 95:e percentilen (3-5 pg TEQ/kg/dag) ligger inom den nedre delen av intervallet 2-10 pg TEQ/kg/dag. Studier av dioxiners kinetik bland barn pekar mot att de något högre intagsnivåerna bland barn än bland vuxna åtminstone till viss del kompenseras av den snabba längd- och viktstillväxt som barn har jämfört med vuxna.

I djurförsök var alltså cancer den hälsoeffekt som uppkom vid de lägsta dioxinexponeringarna när djuren exponerats efter födseln. Mot bakgrund av nuvarande kunskaper så motsvarar ett intag av dioxiner på 2-10 pg TEQ/kg kroppsvikt/dag en mycket liten eller obefintlig cancerrisk. Majoriteten av befolk-ningen i Sverige har dioxinexponeringar som ligger under eller endast något högre än det mest konservativa TDI:t (2 pg TEQ/kg/dag). Detta beror bland annat på att konsumtionen av fet fisk från dioxin-förorenade områden i allmänhet är mycket låg. Halterna av dioxiner i fet fisk från förorenade områden i Sverige är dock fortfarande höga. Det kan därför även i framtiden vara motiverat att informera högkonsumenter av denna typ av fisk om möjligheterna att sänka exponeringen för dioxiner utan att sänka den totala fiskkonsumtionen. Den mest effektiva och långsiktiga åtgärden för att säkerställa tolerabla dioxinhalter i livsmedel är att åtgärda kvarvarande utsläpp av dioxiner från primära och sekundära källor.

Summary

The aim of the present risk assessment was to estimate the tolerable intake level of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs), in the text called dioxins, among humans that will not undergo a pregnancy, i.e. boys, men and post-menopausal women. The risk assessment will be used in the ongoing revision of advisories regarding dioxin-contaminated fish issued by the Swedish National Food Administration. Currently accepted tolerable weekly or monthly intakes of dioxins (TWI/TMI), as developed by the Scientific Committee on Food (SCF) of the European Union (EU) and the FAO/WHO expert group the Joint Expert Committee on Food Additives (JECFA), are based on toxic effects observed in offspring of female rats that were exposed during pregnancy. These developmental TWI/TMI can be used in a revision of consumption advisories regarding girls and women of child-bearing age, but are not applicable for the rest of the human population.

The risk assessment of non-developmental dioxin exposure is one of several scientific inputs to the revision of fish consumption advisories. The weighing of health risks connected to fish consumption against the health benefits of fish consumption is an important part of the advisory revision, and we believe that our risk assessment will give a valuable contribution to this risk-benefit analysis.

Studies of animals exposed to the most toxic dioxin 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) and studies of populations accidentally exposed to dioxins, have shown that the fetal period is the most sensitive life stage. The currently available toxicological literature on non-developmental TCDD exposure suggests that cancer is the most sensitive adverse effect of chronic exposure. In studies of cancer effects during life-time exposure of rats of both sexes, the highest tissue levels of TCDD not resulting in increased risks of tumours was estimated to 540-2000 pg/g lipid (No Observed Adverse Effect Level, NOAEL). As a comparison, the tissue levels of TCDD at the NOAEL for reproductive effects on rat offspring exposed during fetal development was estimated to approximately 130 pg/g lipid by SCF and JECFA.

We used the lipid concentrations of TCDD in the assessment of TCDD body burdens, in contrast to the SCF and JECFA risk assessments, where body burden was expressed as amount of TCDD per kilo body weight. We assumed that the lipid-soluble dioxins at relevant doses are evenly distributed in the different lipid compartments of the body tissues at steady state. The resulting TCDD levels in

lipids give the best estimate of the TCDD exposure of the target organs. In the calculations of TCDD intakes corresponding to the lipid levels of TCDD, the percentage of body fat in rats and mice was set to 10 % and in humans to 20 %. Moreover, we used an intestinal absorption efficiency of 80 % for TCDD, instead of the 50 % absorption that was used by SCF and JECFA. Finally we used a half-life of 7.5 years in humans, which is similar to the assumption made by SCF and JECFA.

TCDD is considered to be a non-genotoxic, yet potent tumour-promoting carcinogen. A threshold approach was therefore used in the risk assessment. In this case, it can be expected that no cancer risk occurs when the exposure level is below the practical threshold of the dose-response curve. We used benchmark modelling to estimate the benchmark dose (BMD) that causes a cancer frequency of 5 % above the background in the rat cancer studies (BMD05). The lower 95 % confidence bound for the BMD05 (BMDL05) was used in the calculation of tolerable daily intakes (TDI) of dioxins. We expressed the tolerably intake on a daily basis in order to simplify the comparison with the long-term daily intakes estimated from Swedish food consumption surveys. In this case the daily intake represents the average long-term intake level of dioxins among in the studied populations. The cancer study performed by the National Toxicology Program in the USA (NTP 2004) was identified as the study with the highest scientific quality. Based on data on liver cholangiocarcinoma in female rats we modelled the BMDL05 to 1500 pg TCDD/g lipid, which corresponds to an estimated human daily intake of TCDD of 95 pg/kg body weight.

In order to arrive at a tolerable intake of TCDD for humans, uncertainties in the assessment need to be accounted for by the use of assessment factors that are applied to the BMDL05. We present three different scenarios for calculations of a dioxin TDI. In the scenarios, different assessment factors were used, to account for uncertainties regarding differences in toxicokinetics and toxicodynamics among humans and to account for the severity of effect. In all scenarios, no assessment factor was required to account for toxicokinetic differences between experimental animals and humans, since TCDD levels in body lipids was used to scale doses across species.

In scenario 1 the default assessment factor of 3.2 was applied in order to account for possible individual differences in toxicokinetics among humans. Similar to the risk assessments performed by the SCF and JECFA, assessment factors for the differences in toxicodynamics between animals and humans and within the human population were not used. SCF and JECFA motivated the omission of these assessment factors on the assumption that the most sensitive humans are not more sensitive to effects of TCDD than the experimental animals. The resulting TDI in scenario 1 after the use of the toxicokinetic assessment factor was 29.7 pg/kg body weight/day.

We do, however, believe that an assessment factor for the differences in toxicodynamics among humans should also be applied to the BMDL05. Mechanistic and epidemiological studies strongly suggest that humans are not more sensitive than rats to cancer effects of TCDD. However, these studies also suggest that there is a substantial individual variation in TCDD sensitivity among humans. It is difficult to separate the contribution of dynamics and kinetics to differences in human sensitivity to dioxins. In scenario 2, we therefore used the default assessment factor for toxicodynamics differences among humans (3.2) in addition to the assessment factor for differences in kinetics among humans (3.2). The resulting TDI in scenario 2, after the use of the total assessment factor of 10, was 9.5 pg/kg body weight/day.

In scenario 3, an additional assessment factor of 5 was used, accounting for the seriousness of the cancer effect. This additional factor may also be motivated since the BMDL05 represents a TCDD dose level that may cause a significant increased cancer risk above background in rats. Moreover, in the critical cancer study on rats, non-neoplastic effects (that may or may not lead to cancer or other severe effects) were observed at dose levels below the BMDL05. The resulting TDI in scenario 3, after the use of the total assessment factor of 50, was 1.9 pg/kg body weight/day.

It was acknowledged that the TDI for TCDD is applicable to other PCDD/DFs and dl-PCBs as long as they are included in the Toxic Equivalency Factor (TEF) scheme developed by the WHO. With the use of TEFs it is possible to estimate the total exposure to Toxic Equivalents (TEQs) of PCDD/DFs and dl-PCBs.

The available epidemiological data on cancer risks in humans obtained from cohorts occupationally exposed to TCDD and cohorts exposed to background levels of dioxins, strongly suggest that the human cancer risks are very low or non-existent at exposure levels in scenarios 2 and 3 (2-10 pg TEQ/kg body weight/day). In the most recent population-based Swedish intake calculation of PCDD/DF/PCB TEQ for adults of ages 17-75 years, the median intake (1.1 pg TEQ/kg body weight/day) was below the most conservative TDI of 2 pg/kg body weight/day. The intake for 95 % of the population was equal to or lower than 2.9 pg TEQ/kg body weight/day, which is only slightly above the most conservative TDI.

Median intakes of TEQ in the population of Swedish children in ages 4-12 years has been estimated to 1.2-2.3 pg TEQ/kg body weight/day, with higher intakes for younger children. The 95 percentile intake was estimated to 3-5 pg TEQ/kg body weight/day, depending on age. These intake levels are below or within the lower TDI range for scenarios 2 and 3. Kinetic modelling of relationships between dioxin intake levels and lipid levels of dioxins in children indicate that the slightly

higher intake levels among children than among adults, are compensated for by the rapid body growth of children. In addition, the developmental of dioxin-related cancer is likely to be a long-term multi-step process during a lifetime of exposure. The slightly higher exposure levels among children are of short duration from a lifetime perspective.

In conclusion, cancer was the most sensitive endpoint for non-developmental dioxin exposure. Based on current scientific knowledge, a TDI range of 2-10 pg TEQ/kg bw/day represents exposure levels where human cancer risks are very low or non-existent. The majority of the Swedish population have long-term dioxin exposure levels that are lower or only slightly higher than the most conservative TDI for non-developmental dioxin exposure (2 pg TEQ/kg bw/d). Concentrations of dioxins in certain types of fatty fish from the Baltic Sea are however still high, i.e. above the maximum limit set for dioxins by the European Union. The con-sumption of these types of fish is low among the general Swedish population. There may nevertheless still be a need for consumption advisories regarding these types of fish, in order to inform consumers, who have a very high consumption of these types of fish, on possible measures to decrease their dioxin exposure. Before final conclusions can be made about how consumption advisories should be formulated, a health-based risk-benefit analysis of high consumption of dioxin-laden fish is needed. The most effective long-term and sustainable measure in order to ensure tolerable dioxin levels in food is to control dioxin emissions from important primary and secondary sources.

Introduction

The term ”dioxins” is used to describe a group of polychlorinated organic chemicals that includes certain polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and co-planar polychlorinated biphenyls (PCBs). These compounds are structurally related, and have been shown to cause toxic responses similar to those caused by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most potent congener within these groups of compounds.

In general, dioxins have very low water solubility, high lipophilicity, low vapour pressure and adsorb strongly to particles and surfaces. They are resistant to chemical degradation, persistent in the environment and bio-accumulate into the biota and are magnified up the food web.

Commercial mixtures of PCBs (e.g. Arochlor and Kanechlor) were widely used as industrial products with diverse applications until 1972, when open use was banned in Sweden. A total ban for all use of PCB has been in place since 1995. However, PCBs may still be present in e.g. old building sealants. In contrast, PCDD/Fs are mainly formed as unintentional by-products during incomplete combustion processes. Recent Swedish source inventories show that the metal, cement, wood and chemical industries, as well as different kind of combustion establishments (e.g. power plants) are important point sources of PCDD/Fs [1]. However, the use of more efficient technology has decreased the emissions from industries and incinerators. Instead, uncontrolled waste burning and secondary emissions from polluted areas and products (e.g. PCP-treated wood) have increased in importance as emission sources. Human exposure to dioxins can occur through occupational exposure in industries in which dioxins are formed as by-products, from industrial accidents, and through intake from food (including breast milk). Exposure via inhalation and dermal absorption is likely to be a minor source for the general public, who are mainly exposed through dietary intake with food of animal origin being the major source. Some subpopulations may have a higher exposure as a result of particular consumption habits (e.g. individuals who consume large amounts of fish from contaminated waters).

Toxic equivalency factors

The PCDDs, PCDFs and PCBs are made up of two linked benzene rings and may have up to 8 or 10 chlorine atoms attached. Each individual compound is referred to as a congener. PCDD/F congeners without chlorine atoms substituted in the 2, 3, 7 and 8 positions are thought to be of no toxicological significance. In total, there are 17 PCDD/F congeners that are thought to pose a health and environmental risk. Like the PCDD/Fs, the biological effects of the PCBs are dependent both on the degree of chlorination and on the position of the chlorine atoms. PCBs that have chlorine atoms substituted to the *para* positions and not more than one *ortho* position can become co-planar, and exert dioxin-like effects in biological systems. In total, there are 12 such PCB congeners. PCDD/Fs and PCBs are always found as complex mixtures in environmental samples. This complicates the risk assessment. However, the potencies of different dioxins have been ranked relative to TCDD, and toxic equivalency factors (TEFs) have been assigned to each congener. To be included in the TEF scheme, a compound must be 1) structurally related to TCDD, 2) bind to the Aryl hydrocarbon Receptor (AhR), 3) elicit AhR-mediated biochemical and toxic responses and 4) be persistent and accumulate in the food web. To estimate the toxic potency of a given dioxin mixture, the mass concentration of each individual congener is multiplied by its TEF, and the products are summarized to a TCDD toxic equivalent concentration (TEQ). In this document we apply the human TEF scheme that was derived in Stockholm on 15-18 June 1997 [2], although we are aware of the re-evaluation of the TEF scheme that was published in 2006 [3]. We agree that other compounds, e.g. brominated dibenzodioxins, may fulfil the criteria to be included in the group of ”dioxins”. However, in accordance with the decision on toxic equivalency factors (TEFs) by WHO in 2006, at present there is insufficient environmental and toxicological data available.

Other national/international risk assessments

Risk assessments performed by the WHO

In May 1998, an international consultation on the assessment of the health risk of dioxins was organized jointly by WHO-ECEH and IPCS. The meeting resulted in a re-evaluation of the TDI and produced a document with a thorough scientific evaluation of all available data [4]. In the assessment, the body burden was for the first time used as the dose metric and the most sensitive adverse responses (LOAELs) reported in experimental animals (developmental effects on reproductive organs, immune suppression and neurobehavioral effects in offspring and endometriosis) were associated with estimated human daily intakes of 14-37 pg/kg body weight (bw)/day. By applying an assessment factor of 10 to this range,

a TDI of 1-4 pg TEQ/kg was established. The upper range was considered to be a maximal intake level and the lower range as the ultimate goal.

In June 2001, a meeting of the joint FAO/WHO expert committee on food additives (JECFA) was held with the purpose of evaluating dioxins [5]. Developmental toxicity was considered to be the critical effect. The committee identified, in addition to the pivotal studies on developmental effects used in 1998, two recent studies [6, 7]. All studies except one were made with single oral bolus doses during gestation. Placental transfer of dioxins may differ between bolus and repeated dose regimen. JECFA therefore used data from Hurst et al. [8, 9] to calculate how much of a higher fetal concentration was obtained from a bolus dose as compared to repeated doses. The data indicated a ratio of fetal to maternal body burden of 1.7-2.6 times higher from a bolus dose. The lowest LOAEL and NOAEL were associated with an estimated human daily intake of 21 and 11 pg TCDD/kg body weight/day, respectively. Assessment factors of 9.6 and 3.2 for LOAEL and NOAEL, respectively, were used. It was decided to express the tolerable intake as a monthly value (TMI) and the range was found to be 40-100 pg/kg bw/month. The committee chose to use the midpoint of this range, 70 pg/kg/month, for the TMI (= TDI of 2.3 pg/kg bw/day).

EU´s risk assessment of dioxins and dioxin-like PCBs in food

In 2000 the Scientific Committee on Food of the European Union Commission released an opinion on risk assessment of dioxins in food [10]. The committee arrived at a temporary TWI (t-TWI) of 7 pg/kg bw for 2,3,7,8-TCDD. It was concluded that the t-TWI could be extended to include all 2,3,7,8-substituted PCDDs and PCDFs, and the dioxin-like PCBs. The evaluation of dioxin toxicity was based on the WHO evaluation of 1998 [4], with an expansion of the database to include studies published after that assessment. Adverse effects in animals were used as a base of the t-TWI calculation, including effects on the reproductive organs and immune system in offspring of TCDD-exposed rats [11-13], effects on development of endometriosis in rhesus monkeys [14] and effects on the learning behaviour of the offspring of TCDD-dosed rhesus monkeys [15].

An assessment factor of 3 was used to account for the use of Lowest Observed Adverse Effect Levels (LOAELs) in the derivation of t-TWI. SCF stated that a factor for toxicokinetic differences between animals and humans was not required, since body burdens were used to scale doses across species. The default assess-ment factor 3.2 was used to account for differences in toxicokinetics among humans. Finally, regarding toxicodynamic differences between animals and humans, it was concluded that studies of the Ah receptor suggest that humans are less sensitive to 2,3,7,8-TCDD than responsive rodent strains. Studies of some biochemical or cellular effects suggest a comparable sensitivity. It could therefore not be excluded that the most sensitive humans might be as sensitive to the

adverse effects of 2,3,7,8-TCDD as experimental animals. No assessment factor was used for extrapolation of differences in toxicodynamics between animals and humans.

In 2001, however, SCF revised its t-TWI to 14 pg/kg bw and removed the designation of temporary [16]. Since the developmental effects were based on studies in rats given single gavage doses of TCDD, an extrapolation was made between body burdens of TCDD after single doses in pregnant rats to body burdens after subchronic gavage exposure. Moreover, the studies on rhesus monkeys were not used in this revision, mainly because of a new study showing that TCDD-exposed animals also had elevated levels of 1,2,3,6,7,8-HxCDF, 3,3´,4,4´-tetrachlorobiphenyl (CB 77) and 3,3´,4,4´,5-pentachlorobiphenyl (CB 126) [17]. In this study increased serum concentrations of 2,3,7,8-TCDD was not associated with the presence of endometriosis in the monkeys, whereas levels of CB 77 and CB 126 were higher in TCDD-treated animals with the disease [17]. Thus, the new TWI was solely based on studies of *in utero* TCDD exposure to rats, mainly a study dealing with decreased sperm production and altered sexual behaviour in TCDD-exposed male rat offspring, both exposed *in utero* and during lactation [6, 16]. The same assessment factors as in the previous assessment were used on the LOAEL (9.6-fold) [16].

UK Food Standard Agency advisory on fish consumption

Regarding the risk of dioxin in other groups of the population than those who have the possibility of pregnancy, the UK FSA Committee on Toxicology performed a risk assessment in 2004 [18]. The EU TDI is set to protect the most sensitive individuals, i.e. the fetus and pregnant woman. Other populations, particularly women past child-bearing age and men, are not at risk regarding developmental effects and are likely to be less susceptible to dioxin toxicity; therefore an alternative TDI was proposed for these groups.

Increased cancer risk was considered the most sensitive and relevant non-developmental effect of dioxins that could be used for risk assessment. Available human data were not considered adequate to identify safety guidelines. The excess cancer mortality in the heavily exposed industrial cohorts was small, and any increased risk of cancer at background levels of exposure was likely to be extremely small and not measurable by current epidemiological methods.

The data from experimental animals were therefore used to recommend a Guideline Level of daily intake over a lifetime that would not be associated with an appreciable risk of cancer. In the Kociba study [19] there was a NOAEL for tumours of 10 ng/kg bw/day of TCDD and of 1 ng/kg/d for hepatocellular nodules. Since TCDD is considered to have a non-genotoxic mechanism of carcinogenicity, the safety assessment was based on the lesion seen at the lowest

dose in the target tissue for tumour development, i.e. the hepatocellular nodules. The interval between dose levels was larger than is now considered appropriate for carcino-genicity studies, and therefore derivation of the Guideline Level from the LOAEL of 10 ng/kg bw/day (and not NOAEL) was considered justifiable.

In deriving the TDI, the COT applied an adjustment factor (3.2) for variability in accumulation of different dioxin-like compounds and a factor of 3 for the use of the LOAEL. Applying this combined adjustment factor of 9.6 (3x3.2; i.e. the same as was used by EU/SCF) to an estimated body burden of 152 ng/kg at LOAEL resulted in a guideline body burden of 16 ng/kg. This guideline body burden was converted into a Guideline Level for a long-term average intake of 8 pg TEQ/kg bw/day, assuming a bioavailability of 50 % and a half-life of 7.5 years in humans.

TDI and risk-benefit analysis

The final result of a risk assessment of contaminants in food is traditionally a TDI that represents a safe level of exposure for humans. In the development of advisories for consumption of dioxin-contaminated fish, a TDI can be used to estimate a level of fish consumption that can be regarded as safe. There may, however, be beneficial health effects connected to consumption of fish for certain groups of people. It may therefore be argued that the potentially positive health effects of fish consumption should be taken into consideration in the risk assessment of contaminants present in fish. It must however be noted that dioxins and dioxin-like PCBs are not only contaminating fish. All foodstuffs of animal origin is contaminated with dioxins. Among Swedish adults on average 50 % of the dioxin intake originates from other foodstuffs than fish [20]. Our risk assessment is focussing on the contaminant and not on the foodstuffs, which contain the contaminant. We have therefore not weighed possible positive health effects of fish consumption into our risk assessment.

A risk-benefit analysis of consumption of dioxin-contaminated fish should, however, be performed during the final development of the fish consumption advisory. In risk-benefit analysis the possible health caused by consumption of dioxin-contaminated fish by a certain risk group is weighed against possible health benefits in the same group caused by the fish consumption. An example of the outcome of a risk-benefit analysis is the conclusion by WHO that breastfeeding should be encouraged since the health benefits of breastfeeding of infants outweigh the risk caused by the dioxin exposure from breast milk [21].

Maximum levels and fish consumption advisories

In 2002, maximum levels of PCDD/DFs were implemented within the European Union [22]. The SCF risk assessment of 2001 was the scientific basis for this risk management decision [16]. In the legislation it was stated that the maximum level should be fixed, taking account of background contamination of foodstuffs within the EU, at a strict but feasible level. It was also concluded that measures based solely on establishing maximum levels for dioxins and dioxin-like PCBs in foodstuffs would not be sufficiently effective in reducing human exposure to dioxins. To be effective, levels have to be set so low that a large part of the food supply would have to be declared unfit for human consumption [22]. Therefore an approach including action levels and target levels in combination with measures to limit emissions were proposed. Target levels would indicate the levels to be achieved in order to ultimately bring exposure of the majority of the population down to the TDI set by the SCF [16, 22].

In connection to the establishment of maximum limits for PCDD/DFs in food, Sweden and Finland were granted a derogation from the maximum PCDD/DF levels for fish, due to fear that exclusion of fish from the diet may have a negative health impact in these countries [22]. In these countries, fish from the Baltic region with PCDD/DF levels above the maximum limit can be placed on the domestic market. It was concluded that the derogation was given since Sweden and Finland have a system in place (i.e. consumption advisories), which has the capacity to ensure that consumers are fully informed of the dietary recommend-dations concerning restrictions on consumption of dioxin-contaminated fish from the Baltic region [22]. The dioxin concentrations in certain fat-rich wild fish species from the Baltic Sea, Bothnian Bay, Lake Vänern and Lake Vättern are high in comparison to levels normally found in farmed and wild fish sold on the Swedish market [23].

From November 2006, maximum levels for dioxin-like PCBs are included in EU regulations [24]. The Swedish and Finnish derogation from maximum limits in fish have been changed to be applicable for salmon (*Salmo salar*), herring (*Clupea harengus*), river lamprey (*Lampreta fluviatilis*), trout (*Salmo trutta*), char (*Salvelinus spp.*) and roe of vendance (*Coregonus albulas*) originating in the Baltic Sea region. The derogation from maximum levels has been authorised for a transitional period to the end of 2011. The prerequisite of fish consumption advisories for contaminated fish is still applicable [24].

In Sweden, consumption advisories regarding fish contaminated with organo-chlorine compounds have been in force since the early 1980s. The current advisory recommends that consumers eat fish 2-3 times a week, including one meal of fatty fish per week [25]. Fatty fish from certain areas are, however, contaminated with dioxins and PCBs. The current advisory that was published in 1995 recommends girls and women of child-bearing age to consume herring and wild salmon/trout from the Baltic Sea and the Bothnian Bay, wild salmon/trout from Lake Vänern and wild salmon/trout/arctic char from Lake Vättern on average not more than once a month. Other consumers are advised to consume these fish not more than once a week [26].

Toxicokinetics

The toxicokinetics of dioxin and related compounds seem to depend on three major properties: lipophilicity, metabolism and binding to CYP1A2 in the liver. Lipophilicity increases with chlorination and controls absorption and tissue partitioning. Metabolism is the rate-limiting step for elimination, and induction of CYP1A2 (via AhR) leads to a hepatic sequestration of certain congeners. Kinetic data are essential to the risk assessment when experimental results are extrapolated between species and within the same species. However, many congeners lack kinetic information and this hampers a proper risk assessment of dioxin in complex samples.

Uptake and distribution

Human exposure to PCDD/Fs and PCBs is mainly due to oral ingestion. The available literature does not indicate major interspecies differences in absorption of dioxins. Molecular size and lipophilicity are the rate limiting factors for the absorption from the gastrointestinal tract. For example, octa-chlorinated PCDD/Fs are reported to have a lower bioavailability than PCDD/Fs with less chlorine substitution [27, 28]. Also the absorption of PCBs seems to depend mainly on molecular size [29] [30].

The WHO and the EU-SCF report a fractional absorption of 50 % with reference to two studies on rats [19, 31] and one study on cows [32]. The US-EPA also sets 50 % as the gastrointestinal absorption level, although for unclear reasons. These absorption values are significantly lower than those reported from most other kinetic studies on dioxin-like compounds. Most studies indicate an absorption of between 60 and 95 % of the given dose, partly depending on the lipid content of the food. Similar values are reported for rats [8, 9, 29, 31, 33-35] nursing infants [36-38] and human volunteers [39, 40]. We therefore used 80 % as a fractional absorption constant in the calculation of tolerable intakes.

Once dioxins have been absorbed from the gastrointestinal tract, the bodily distribution of the compounds is initially determined by the binding capacities of blood components and the perfusion rate of the tissues. As a result, high initial concentrations have been observed in liver and highly perfused tissues in animals exposed to PCDD/DFs [30, 41]. After initial distribution, the adipose tissue and liver are the predominant storage sites of dioxins. However, at high doses, binding to hepatic CYP1A2 protein results in extra sequestration (up to 70 % of body

burden) of dioxins in the liver [42]. At low constant doses there is a dynamic steady state with constant partition coefficients between the body compartments. However, in humans as well as in laboratory animals, the portion of adipose tissue varies over time. For most people it increases with age, which may result in a dilution of already accumulated dioxins.

Metabolism and elimination

The liver has been identified as the principal organ for PCDD/F- and PCB-metabolism. The metabolism of xenobiotics, including PCDD/Fs and PCBs, generally results in the formation of more polar metabolites, which are subsequently cleared from the organism. Several hydroxyl- or methylsulfonyl-PCB metabolites have been identified and have been found to be retained in human tissues. In addition to metabolism, there is an excretion of contaminants from the body's reservoirs via the faeces. Breast-feeding has also been shown to decrease the maternal body burden.

As a result of the low metabolic conversion rate, the half-lives of PCDD/Fs and PCBs are generally quite long. Mean half-lives of TCDD have been reported to vary from 5.1 to 11.3 years in occupationally exposed males [43], 5.8 years in a voluntary male and approximately 7 years in Ranch Hand personnel [41]. The three most abundant PCDD/Fs (expressed as TEQ contribution), namely 2,3,4,7,8-PCDF, 1,2,3,7,8-PCDD, and 1,2,3,6,7,8-PCDD, show median half-lives in humans estimated to 20, 16 and 13 years, respectively [43]. The elimination rate seems to be exposure dependent, and after high doses (e.g. after acute TCDD intoxication) a half-life of less than three years has been reported. Transition to non-linear kinetics seems to occur at levels in the blood of about 0,1-1 ng TEQ/g fat [44, 45]. This may be due to enhanced metabolic capacity and/or an increased binding to CYP1A2 in the liver. There is also data showing that half-lives increase with age [46]. This may be due to either a changed body composition or a slower metabolic capacity. It is important to remember that dioxin binding to AhR or CYP1A2 differs depending on the molecular structure of the dioxin congener. There is also large interspecies variation in the half-lives of dioxins. In laboratory animals (mostly rats), the half-lives of PCDD/Fs are found to be much shorter compared to humans. The half-life of TCDD has been reported to range between 11 to 37 days in rats, about 8 days in mice, and about a year in monkeys. There are few half-lives reported for PCBs. In a volunteer study, PCB153 was reported to have a half-life of almost 1 year. Results from Yusho and Yu-Cheng indicate half-lives for highly chlorinated PCBs of about 4 years. In rats, most high-chlorinated PCBs were reported to have half-lives longer than 90 days, whereas low-chlorinated congeners had initial half-lives of 1-2 days [29].

Toxic effects

In this risk assessment, toxic effects in both animals and humans are reviewed. We have concentrated on TCDD-induced effects, but in some cases studies on other PCDD/DFs or dioxin-like PCBs are used. Both non-adverse and adverse effects are included.

In populations with background exposure to organochlorines, mainly from food, correlations between body lipid levels of highly chlorinated PCBs and PCB/PCDD/DF TEQs are strong [47, 48]. Consequently, PCB levels in lipids could be regarded as proxy markers of levels of TEQ. Some of the epidemiological studies where PCB levels have been used in exposure assessment have therefore been included in the risk assessment.

Biochemical effects

The physiological role of the AhR remains elusive, as does that of the endogenous ligands. These issues are a missing key to a more accurate risk assessment of dioxin. Studies using AhR-deficient mice indicate that AhR is necessary for normal development [49-52]. AhR regulates genes with importance for cell proliferation, differentiation and apoptosis. However, the toxic effects mediated via the AhR cannot be fully explained by any of the AhR target genes identified to date. An extensive cross-talk between the AhR and other transcription factors, including NF-κB [53], retinoblastoma protein [54] and the estrogen receptor [55], as well as other ARNT dependent factors such as HIF-1α, may explain some of the toxic effects.

AhR regulates a huge battery of genes. These genes include phase I enzymes like CYP1A1, CYP1A2, and CYP1B1, as well as phase II enzymes such as GST and UDPGT. Additionally, TCDD can influence several genes involved in the control of cell growth such as TGF-α, c-fos and c-jun [56]. Screening with micro-array techniques for altered gene products has clearly identified a wide range of genes that depend on the AhR either for constitutive expression or for response to TCDD [57-59].

Table 1. Sensitive biochemical effect of TCDD in animals according to WHO [4].

Effect	Species	Exposure (LOEL)	Body Burden
CYP1A1	Mouse	150 pg/kg/day	3 ng/kg
	Rat	100 pg/kg/day	3 ng/kg
CYP1A2	Mouse	450 pg/kg/day	10 ng/kg
EGFR	Rat	100 pg/kg/day	3 ng/kg
IL1beta	Mouse	300 pg/kg/day	10 ng/kg

Biochemical responses on metabolising enzymes can be detected at administrated doses in rodents of 100 pg/kg/day (Table 1). This corresponds to body burdens about a factor ten times lower than those causing more adverse effects in animals. However, whether these changes are adverse or adaptive is a matter of discussion. Induction of CYP1A1 is associated with enhanced sensitivity to cancer (e.g. lung cancer among smokers [60]). Biochemical responses may also lead to inappropriate physiological processes (e.g. metabolism of sex hormones during crucial developmental stages).

CYP1A

Whereas CYP1A2 is constitutively expressed in human and rodent liver tissue, CYP1A1 is constitutively expressed at very low levels, but highly inducible in liver tissue, as well as in other tissues including kidney, intestine, lung, skin and the brain. Induction may sometimes be disadvantageous since CYPs have a broad substrate specificity, and induction of the enzyme may lead to increased metabolism by a second substrate, thereby altering kinetic properties of that substrate [61]. In addition, the induction can lead to the formation of reactive metabolites (e.g. epoxides and arene epoxides) that can bind covalently to macromolecules, like DNA, and cause mutagenicity and carcinogenicity. No relation between CYP1A2 induction and TCDD was found when a cohort of chemical workers from the USA with high TCDD exposure (NIOSH cohort) was examined [62]. Caffeine metabolism (measure of CYP1A2 activity) was increased up to tenfold in two TCDD-intoxicated patients with serum lipid TCDD concentrations of 10.000 and 100.000 pg/g [63]. A third subject with a TCDD concentration of about 500 pg/g lipid showed no extra induction, indicating that moderate exposure (up to about 1000 pg/g) does not cause clinically discernable CYP1A2 induction.

GGT

Gamma glutamyl transferase (GGT) activity has been elevated in several occupationally and accidentally exposed human cohorts [64] as well as in long term animal studies [65]. The use of GGT as a marker of TCDD is hampered by

the fact that many hepatobiliary diseases and alcohol consumption both increase GGT activity.

Endocrine effects

There are several effects on humans, which have been suggested to be linked to environmental exposure to endocrine disrupting chemicals present in complex samples like food. These effects include developmental effects, reduced sperm quality and increased cancer incidence [66, 67]. Most of these hypotheses remain to be proven. TCDD interacts with hormone systems at various levels, by altering hormone metabolism (e.g. decrease of melatonin [68]), interfering with hormone receptors (e.g. decrease of glucocorticoid receptors in the liver of rats [69]), and modulating gene transcription.

Vitamin A

Reduction of hepatic vitamin A levels is one of the most sensitive responses to TCDD exposure [70]. The reduced hepatic levels of vitamin A correlate very well with other subchronic toxicological responses [71]. TCDD may also interfere with the retinoid system via competitive binding to co-factors [72] or interference with the retinoic acid receptor/response element binding [73]. A LOEL of 14 ng/kg/day (lowest dose tested) is reported by van Birgelen et al. [74] for decrease in hepatic vitamin A.

Thyroid hormones

Several studies on rats have shown that thyroid hormones are affected by dioxin. Decreased plasma levels of total thyroxine (TT4) and free T4 in rats have repeatedly been reported [75-77]. A NOEL in rats of 26 ng/kg/day for plasma TT4 was reported by van Birgelen et al. [78]. However, the effects on triiodothyronine (T3) levels are more variable. Thyroid stimulating hormone (TSH) levels may be increased due to a compensatory feedback mechanism involving the hypothalamus and pituitary.

Human data from occupationally exposed workers showed in some cases weak correlations between dioxin exposure and effects on thyroid hormone levels, but were in most cases inconsistent [79-82]. Within a German cohort of accidentally exposed workers (BASF), thyroid disease was diagnosed more often than in a referent population [83]. However, no excess in thyroid disease was seen in the cohort of Vietnam veterans exposed to TCDD during the handling and use of the herbicide ”Agent Orange” (Ranch Hand cohort) [84] or in occupationally exposed US workers (NIOSH cohort) [81]. Among examined thyroid-related parameters,

concentrations of T4 and thyroid-hormone-binding-globulin (TBG) were increased within the BASF cohort [80], but not within the NIOSH cohort [81].

In addition to the studies mentioned in JECFA 2001, the following additional studies have been retrieved. Zober and co-workers [83] did a morbidity follow-up of the BASF workers (n=158) earlier studied by Ott et al. [80]. Results showed that thyroid disease was diagnosed more often in the high TCDD subgroup, but that the disease was not differentially distributed by chloracne state.

In a study on workers in a nonferrous metal recycling facility (n=76), PCDD/DF blood levels could not be associated with serum thyroid hormone levels [85].

In patients from a PCDF/PCB poisoning accident in Japan (Yusho, n=16), with markedly elevated TEQ levels (about seven-fold higher than that of healthy Japanese people), TEQ exposure was not associated with serum levels of thyroid hormones [86].

In an Australian study, workers (n=37) exposed to dioxins during 2,4,5-T spraying were followed [87]. A weak negative correlation between TCDD levels and both T3 and TSH levels were found. Possible confounders were not discussed.

As a follow-up of the studies by Grubbs *et al.* [82], a thyroid function study was performed on the Ranch Hand cohort [84]. Various thyroid hormone parameters were studied in relation to serum TCDD levels. Data was available from 1009 Ranch Hand veterans and 1429 comparison veterans participating in any of five examinations in 1982, 1985, 1987 and 1992. Based on their serum TCDD levels, each veteran was assigned to one of four exposure categories. Statistically significantly increased TSH levels among veterans with the highest TCDD exposure both at the time of the 1985 and 1987 examinations and trends of increased TSH levels at all four examination time-points were observed. There were no significant relation between the occurrence of thyroid disease and the TCDD category.

Several studies have been performed on infants and children [88-95]. Some of these studies suggest associations between levels of thyroid hormones in infants and the dioxin levels in the mothers´ breast milk. However, as the dioxin levels in breast milk mirrors both the body burden of the mother and neonatal exposure during the breast-feeding period, the *in utero* exposure and the breast milk exposure cannot easily be discriminated from each other. In none of the above-mentioned studies was an attempt made to separate these two exposure routes. Since our risk assessment deals with the extra-uterine exposure, these studies were not used by us. Moreover, the concurrent co-exposure to other POPs, including non-dioxin-like PCBs, makes the exposure part problematic to handle.

There are also some examples of non-occupational adult study populations with suggested thyroid hormone alterations as a result of POP exposure. In the study by Koopman-Essebom and co-workers [90], altered thyroid hormone levels (decrease in total serum T3 and T4) in pregnant and nursing women were suggested to be related to dioxin concentrations in breast milk. Effects on thyroid hormones have also been suggested after exposure to non-dioxin-like PCB: Hagmar and co-workers [96] observed a decrease in TT3 with increased PCB-153 concentrations in the blood plasma of Swedish fishermen's wives. Moreover, in the Great Lakes Sport Fish Consumption Study, the levels of serum T4 in participating subjects (including anglers, boat captains and referent participants) were negatively correlated to the blood levels of the sum of 89 PCB congeners [97].

In conclusion, not much new data have been added since earlier assessments [4, 98] and the most important new study may be the follow-up of the Ranch Hand cohort performed by Pavuk and co-workers [84], not least because of the large number of study objects. However, the study design is difficult to understand, and this makes the subsequent interpretation of the observed associations between TCDD exposure and effects on TSH levels hard to evaluate. A few non-occupational studies have reported weak associations between dioxin exposure and thyroid hormonal levels. In these studies, problems with co-exposure to e.g. non-dioxin-like PCBs make it difficult to interpret the results. Moreover, many of the studies have not addressed potential confounder problems. When also taking into account the question of what is considered to be a harmful effect (variations within the "normal" range), it is suggested that the studies on dioxin effects on thyroid hormone homeostasis in adult human populations should not be used in the following establishment of a TDI/TWI. The studies on effects in babies/infants should not be used due to problems with mixed intra- and extra-uterine exposure.

Steroid hormones

Several steroid hormones are affected by dioxin. Serum testosterone and dihydrotestosterone were dose-dependently depressed in TCDD-treated rats [99, 100]. In boys at puberty, who were previously exposed prenatally during the PCDF/PCB poisoning accident on Taiwan (Yu-Cheng), a decrease in serum testosterone was observed together with increasing estradiol and FSH levels [101]. In girls exposed prenatally during the same accident, serum levels of estradiol and FSH were increased at puberty. The girls also showed a shorter duration of menstrual bleeding and irregular menstruation as compared to control subjects.

However, some of the low dose animal studies on male reproduction have also measured levels of testosterone without finding any significant decrease [6, 7,

102]. Dioxin does not cause classical estrogenic responses. Instead TCDD inhibits several 17β-estradiol-induced responses in rodent mammary glands and uterus tissue and in human breast cancer cell lines [103]. However, TCDD does not block estrogen binding to the estrogen receptor (ER), nor does it bind to the ER. Rather, it affects the DNA binding of the liganded ER [55]. Although anti-estrogenic actions of dioxins are well described, dioxins can also induce endometriosis and estrogen-dependent tumours in animals, implying possible estrogenic effects. It was recently shown that the agonist activated AhR/ARNT heterodimer directly associates with ER-alpha and ER-beta [104]. Besides the receptor-mediated effects, the pituitary appears to be a target organ for dioxin, where the normal feedback mechanisms between plasma testosterone/estradiol and LH secretion are disturbed [105]. Dioxin can also alter the metabolism of both estrogens and androgens.

Cancer

The literature on carcinogenic effects of dioxins have been extensively described in the IARC report as well as in the risk assessments of WHO and EU/SCF [4, 10, 16, 106].

Animals

Many studies show that TCDD is not genotoxic, but is a potent tumour promoter in organs, such as liver, skin, lung, palate and thyroid gland. Several tumour promotion studies are available. Mechanistic studies show the involvement of the Ah receptor in tumour development. Dependence of factors such as hormones, cell proliferation and apoptosis has been studied. Besides the full cancer studies, these more mechanistic studies are difficult to use for quantitative risk assessment (i.e. calculating a TDI). Below are presented the two studies where TCDD caused tumours at the lowest exposures reported.

A lifetime cancer study on showed that TCDD in the diet causes cancer. Liver tumours in female rats was identified as the most sensitive effect [19] (see Table 2). Fifty males and fifty females per group were studied at the dose levels of 0, 1, 10 and 100 ng/kg bw and day. The NOAEL was defined to 1 ng/kg bw and day based on non-neoplastic effects. A pathological re-evaluation of the Kociba study [107] revealed that combined hepatocellular adenoma and carcinoma was increased from 10 ng/kg/d, which further strengthens the NOAEL of 1 ng/kg/d. The dose level of 1 ng/kg/d corresponds to a measured concentration of 0,540 ng TCDD per g in fat tissue at the end of the study (2 years).

Table 2. Non-neoplastic effects reported in cancer studies of TCDD [19, 108].

Critical effects (non-neoplastic effects)	**NOEL/NOAEL (ng/kg/d)**	**LOEL/LOAEL (ng/kg/d)**
Kociba (SD rat)		
Liver lesions	1	10
Lung lesions	1	10
Increased urinary excretion of porphyrins (♀)	1	10
NTP 2004 (female Harlan rat)*		
Labelling index in hepatocytes	-	3
Hepatic EROD, PROD, A-4-H activity	-	3
Bronchiolar metaplasia	-	3
Gingival squamous hyperplasia	-	3
Non-neoplastic hepatic changes	3	10
Hepatic necrosis, oval cell hyperplasia, bile duct hyperplasia	10	22
Thymic atrophy	22	46
Adrenal cortex hyperplasia	3	10
Cardiomyopathy	3	10

*dosing 5 days/week

Recently, a large cancer study in female Harlan rats with 80 rats per group was reported [108, 109]. Rats were dosed via gavage five days a week, at dose levels of 3, 10, 22, 46 and 100 ng TCDD/kg bw. At all dose levels (from 3 ng TCDD/kg) an increased labelling index in hepatocytes, hepatic EROD, PROD, A-4-H activity, bronchiolar metaplasia and gingival squamous hyperplasia was reported (Table 2). Non-neoplastic hepatic changes were observed from 10 ng/kg body weight/day. The most sensitive neoplastic effects were liver cholangiocarcinoma and total number of malignant tumours, which were increased at 46 ng/kg/day and above (Table 3). The dose levels 3, 10, 22, 46 and 100 ng/kg corresponded to concentrations of 0.51, 0.75, 1.4, 2.0 and 3.2 ng/g in fat, respectively, at the end of the study.

Table 3. Neoplastic effects reported in cancer studies of TCDD [19, 108].

Critical effects (neoplastic effects)	**NOAEL (ng/kg/d)**	**LOAEL (ng/kg/d)**
Kociba (SD rat)		
♂		
Carcinoma of hard palate or nasal turbinates	10	100
Carcinoma of tongue	10	100
Adenoma of pancreas	10	100
Adenoma of adrenal cortex	10	100
Adrenal pheochromocytoma	10	100
♀		
Hepatocellular nodules	1	10
Combined adenoma and carcinoma (re-evaluation)	1 10	10 100
Hepatocellular carcinoma	10	100
Carcinoma of hard palate or nasal turbinates	10	100
Squamous cell carcinoma of lung		
<u>Decreased frequency:</u>	10	100
Uterus benign tumours	10	100
Benign mammary neoplasm	10	100
Mammary carcinoma	10	100
Pituitary adenoma		
NTP 2004 (female Harlan rat)*		
Liver cholangiocarcinoma	22	46
Hepatocellular adenoma	46	100
Cystic keratinizing epithelioma lung	46	100
Gingival squamous cell carcinoma	46	100
All malignant tumours	22	46
<u>Decreased frequency:</u>		
Mammary gland tumours	46	100
Pituitary gland adenoma	46	100
Thyroid gland tumours	46	100

*dosing 5 days/week

To summarize, the overall NOAEL for adverse effects was in the range of 1-3 ng/kg/d. Higher doses caused increases in combined hepatic adenoma and carcinoma [19, 107] or other pathological effects (Table 2) [108].

Humans

The cancer studies published up to 2000 have been extensively reviewed by IARC [106] and by two WHO expert groups [98, 110]. In 1997 IARC classified TCDD as a human carcinogen [106], based on sufficient evidence on animals and on limited evidence on humans. Human evidence was mainly derived from epidemiological studies on TCDD-exposed occupational cohorts. In the evaluation, mechanistic considerations focussing on the Ah-receptor were taken into account in the final conclusion [106]. The epidemiological studies showed increased risks of all cancers combined in TCDD-exposed occupational cohorts from the US, Germany (BASF and chemical worker cohorts), and the Netherlands. In one of the German cohorts, a positive dose-response trend was shown. Less strong evidence was found for associations between TCDD and risks for cancer at specific sites, such as lung cancer and soft-tissue sarcoma [106].

Table 4. Mean (min-max) TCDD or PCDD/DF TEQ levels in blood lipids in cohorts including individuals with high occupational and/or accidental exposure.

Cohort	N	Levels in lipids (ng/kg)	References
German chemical workers[a]	1189	110 (2-2300)	[111]
USA chemical workers[b]	3444	1600 (6-210000)	[112]
BASF (no chloracne)[b]	139	10 (3-7600)	[113]
BASF (moderate chloracne)[b]	59	720 (3-7000)	
BASF (severe chloracne)[b]	56	1500 (3-12200)	
Dutch (accident)[c]	14	1400 (900-2300)	[114]
Dutch (exposed, no accident)[c]	17	70 (30-175)	
Seveso (Zone A)[d]	296	452 (3-56000)	[115]
Seveso (Zone B)[d]	80	94 (4-1450)	
Seveso (Zone R)[d]	48	55 (2-545)	
Ranch Hand (low)[e]	281	66 (32-119)	[116]
Ranch Hand (high)[e]	282	245 (119-4220)	

[a]Levels at blood sampling on average 14 years since end of employment
[b]Back-extrapolated to the end of exposure to TCDD/PCDD/DF
[c]Back-extrapolated levels in a sub-group of the cohort (N=1167), geometric mean (95 % confidence interval)
[d]50th percentile and 0-100 percentiles shortly after the accident
[e]Median and range extrapolated to end of service in Vietnam

The WHO risk assessments were based on industrial cohorts from Germany, USA, the Netherlands, and Great Britain, on the IARC multi-country industrial cohort, and on the Seveso cohort [98, 110]. It was stated that the associations between high TCDD exposure and increased cancer mortality could most likely not be explained by confounding, but this possibility could not be excluded. The studies were prospective and well conducted. Caution should, however, be taken since the overall relative risks were not high, being 1.4 in most highly exposed and longer-latency sub-cohorts. There are very few carcinogens that affect the risk of all cancers with no clear excess for any specific cancer. Finally, it was concluded that strongest evidence came from cohorts with an exposure that was two to three orders of magnitude higher than that of the general population [98, 110].

In the industrial cohorts, blood lipid TCDD levels at the last time of exposure were back-calculated from TCDD measurements performed many years later in sub-groups of the cohorts (Table 4). For individuals with no serum results available, serum levels were estimated from exposure models derived from the TCDD analysis of sub-groups in the cohorts. Serum TCDD levels were back-calculated using a half-life of 6-9 years [111, 112, 114, 117].

New studies - meta analysis

Recent studies of the association between TCDD exposure and cancer in humans both include extended studies of the industrial cohorts and a few studies of new cohorts. A meta-analysis of data from the US cohort and the two German cohorts showed a statistically significant trend in total cancer mortality with increased dioxin exposure [118].

Seveso

In 2001, a 20-year follow-up of cancer mortality among the populations affected by the Seveso accident was performed [119]. During the entire observation period, total cancer mortality did not increase. In zone A, the risk of mortality in cancer was not increased, but in zone B a significant increased risk was seen for rectal cancer, and lymphatic and haemato-poietic cancers. For females in zone A and B combined, an increased risk for lymphatic and haematopoietic cancers was found, and for men an increased risk for all cancers, rectum cancer, lung cancer and myeloid leukaemia.

In a separate analysis of 981 women in zone A and B, with known or estimated serum levels of TCDD at the time of the accident, breast cancer incidence was significantly related to serum TCDD levels (cases:median 72 pg/g lipid; controls:median 55 pg/g lipid) based on a limited amount of cases (15 cases) [120]. A ten-fold increase in serum TCDD level resulted in a doubling of hazard

rates. When the women were categorized in four exposure groups, no significant dose-response trend was found [120].

Ranch Hand/Agent Orange

A cancer study on the Ranch Hand veterans of the Vietnam war, who had handled and sprayed herbicides contaminated with TCDD during the war, was published in 2004 [116]. The risk of cancer mortality was decreased in both the Ranch Hand cohort and a comparison cohort of veterans not exposed to Agent Orange, as compared to the national rate. The incidence of all cancers was not increased compared to the national rates in Ranch Hand and comparison cohorts. The incidence of prostate cancer and cancer at ill defined sites was significantly increased in the comparison cohort and cancer of the urinary system and lympho-poetic system was significantly decreased compared to national rates. Among white Ranch Hand veterans a significantly increased risk of cancer at any site was found for the Ranch Hand high category when compared to the Ranch Hand comparison group. The risk of melanoma was increased in both the low and the high category and the risk of prostate cancer was increased in the high group [116].

Two case-control studies on the risk of prostate cancer among veterans suspected to have been exposed to Agent Orange did not find any statistically significant associations between cancer and exposure to the herbicide [121, 122].

Soft-tissue sarcoma in Finland

The association between soft-tissue sarcoma (STS) and individually analysed dioxin exposure was studied in a general population exposed to relatively low levels of dioxin from food [123]. A prospective case-control study was performed in 16 hospitals in southern Finland, including 110 cases of STS and 227 area- and age-matched controls operated for appendicitis. The average PCDD/DF concentration among the participants was 33 pg TEQ/g adipose lipid (range:1-146 pg/g lipid). The STS risk did not increase with increasing body burdens of dioxin TEQ. Instead, the risk appeared to be highest in the study participants with the lowest PCDD/DF body burdens. The same trend was confirmed after also including non-*ortho* PCBs [124].

Breast cancer in California, USA

In a hospital-based case-control study, 79 cases with histologically confirmed invasive breast cancer and 52 controls with benign histological changes were sampled for analysis of PCDD/DFs in adipose tissue [125]. This small study could not find any statistically significant associations between breast cancer risks and adipose tissue levels of PCDD/DFs [125].

Non-Hodgkin's lymphoma in the US

A population-based case-control study of non-Hodgkin's lymphoma included 100 untreated cases and 100 matched controls that were selected from a larger case-control study [126]. The random selection of cases and the selection of matching controls were done among study participants that had donated enough blood volume to allow PCDD/DF analysis. A statistically significant positive trend of association was found between cancer risk and total TEQ levels in blood plasma. When looking at PCDD, PCDF and PCB TEQs separately, only the trend for PCDF TEQs was statistically significant [126]. When comparing different exposure quartiles, none had an odd ratio that was significantly different from the reference quartile. A positive association between risk of non-Hodgkin's lymphoma and certain non-dioxin-like PCBs was also found [126]. Lipid-adjusted plasma levels of dioxins were used in the statistical analysis, and the cases had lower levels of plasma lipid than the controls (<10 %). It was proposed that this difference in lipid levels could be disease-related or due to transient changes resulting from feeding patterns during the day before the blood was drawn [126]. It was concluded that without further knowledge about the biological changes post-diagnosis, it is impossible to determine whether the results are affected by bias. The total TEQ levels in plasma lipids were reported to range from ≤15 to>29 pg TEQ/g lipid.

Swedish fishermen families

Fishermen living on the Swedish east coast have a high consumption of fatty fish from the Baltic Sea [127, 128]. In 2002 blood from nine fishermen was analysed and the median level of PCDD/DFs was 65 pg TEQ/g lipid (min-max: 12-170), and median of non-*ortho* PCB TEQ was 17 pg/g lipid (4-77) [127]. Among 2896 east coast fishermen the incidence of squamous cell skin and stomach cancer was increased in comparison to the regional general population, with a standardized incidence ratio (SIR) of 1.6 (95 % confidence interval: 1.0-2.4) for stomach cancer and 2.3 (1.5-3.5) for squamous cell skin cancer [129]. Incidence of colon cancer was decreased. Mortality of multiple myeloma was higher among east coast fishermen (standardized mortality rate: 3.1 (1.2-6.4). No exposure assessment of dioxins or other related compounds was done, and confounding due to differences in life style was not accounted for. It was concluded that the increases in stomach and skin cancer could not with any certainty be attributed to exposure to organo-chlorine contaminants, since the fishermen were also exposed to other potentially carcinogenic compounds (arsenic, compounds in smoked fish) [129].

Other studies

A few new studies have been published that have not assessed the dioxin exposure by measuring dioxins in either the study participants or in sub-groups of study participants. Phenoxy herbicide producers and sprayers in New Zealand were followed up for 27-31 years [130]. A total of 813 producers and 699 sprayers were classified as having been exposed to dioxins and phenoxy herbicides. The results showed a non-significant excess (24 %) in cancer mortality and a significant excess for multiple myeloma when standardised mortality ratios were calculated using national mortality rates. Dioxin levels among the study participants were not measured, so it is not possible to draw conclusions about associations between disease and exposure.

In two case-control studies from Italy and France, with indirect exposure assessment, an increased risk of STS was found among individuals living near an incinerator [131] and an increased risk of non-Hodgkin's lymphoma was found among those living in an area with the highest environmental dioxin concentrations [132]. No measurements of dioxin exposure levels were done, making it impossible to draw conclusions about associations between dioxin exposure and risk of disease.

In populations with background exposure to organochlorines, mainly from food, correlations between body lipid levels of highly chlorinated PCBs and PCB/PCDD/DF TEQs are high [47, 48]. Consequently, PCB levels in lipids could be regarded as proxy markers of levels of TEQ. Associations between background PCB exposure and breast cancer among women have been extensively studied. Several reviews have summarised the prospective and retrospective studies published, concluding that the epidemiological evidence does not support the hypothesis of an association between background exposure to PCBs in adulthood and risk of breast cancer [133-135]. A few studies, however, indicate that certain highly exposed sub-groups of women with certain genotypes of the polymorphic CYP1A1 gene may be at risk [136-139].

Conclusions

Taken together the epidemiological studies cannot be used in the calculation of TDIs for dioxin exposure. They can nevertheless be used to estimate the highest possible risk, assuming that the risk of cancer is causally associated with dioxin exposure in the occupational cohorts (see Discussion). These estimates are, however, uncertain because of the reasons mentioned below.

In the occupational cohorts, co-exposure to other chemicals than TCDD has rarely been accounted for. Many of the workers were exposed to TCDD as a contaminant during production and/or handling of both halogenated and non-

halogenated compounds. For instance, the German chemical workers came from different departments involved in the production of and handling of trichlorophenol, 2,4,5-trichlorophenoxyacetic acid, bromophos and hexachlorocyclohexane (including lindane) [111]. Furthermore, apart from a substantial exposure to the chlorinated chemicals produced and handled in the different departments, the workers were most likely exposed to solvents and other chemicals needed in the synthesis of the chemicals and formulation of the end products. Whether this multiple co-exposure contributed to the observed associations between TCDD and cancer is not known.

Another problem with the epidemiological studies is the lack of TCDD analysis in blood lipids or adipose tissue among a large number of study participants. In most of the occupational studies, TCDD levels in body lipids have been modelled from analytical results in sub-groups of the studied cohorts and individual job histories [111, 112, 114]. In the IARC study, exposure assessment was based on individual job records, company exposure questionnaires and in some sub-cohorts, measurements of TCDD and other PCDD/DFs in body lipids [140]. In a cohort involved in chlorophenoxy herbicide manufacturing, which was included in the IARC study, TCDD and PCDD/DF levels in a random sample of 20 employees were only slightly elevated in comparison to background levels [141]. It was concluded that PCDD and PCDF exposure of cohort members was unlikely to explain the elevated SMRs for several cancer sites observed in this cohort [141]. In the Seveso study, exposure assessment was based solely on the place of residence at the time of the accident (exposure: zone A>zone B>zone R) [119].

The exposure level during and directly after the occupational/accidental exposure is also uncertain for another reason. In recent studies of TCDD elimination kinetics in humans from Seveso, Italy, Vienna, Austria, the US (Ranch Hand cohort) and Germany (BASF cohort), it has been suggested that the use of half-lives of 6-9 years in back-calculation of serum levels in occupational cohorts may have underestimated the serum levels at the time of exposure [44, 45, 142]. In highly exposed cohorts half-lives were both age- and concentration-dependent, with shorter half-lives at high concentrations and younger ages [142].

Cardio-vascular effects

Animals

Short-term high exposures of animals to TCDD cause alterations in lipid metabolism in the liver, which subsequently could change the levels of triglycerides and cholesterol in the blood [143]. High TCDD doses may also cause oedema and alter cardiac function and morphology in several species [143]. Mechanistic studies have shown that TCDD may cause changes in gene regulation

and biochemistry in the cardio-vascular system, which could be markers of heart disease [144, 145]. High exposure of C57Bl/6J mice to TCDD (5 μg /kg/d for three days) caused increased mean tail-cuff blood pressure [144]. Similarly, high exposure of female Sprague-Dawley rats to the dioxin-like PCB congener CB 126 for 5 weeks (total dose 224 μg/kg) caused increased blood pressure [146].

In male marmoset monkeys treated with a single subcutaneous dose of 100 ng TCDD/kg (n=10) significantly more collagen was detected in the left ventricular myocardium in comparison to vehicle treated control animals (n=13) [147].

In a National Toxicology Program investigation of chronic effects of TCDD, female Harland Sprague-Dawley rats (n=50-53 per group) were exposed via gavage five days a week to 3, 10, 22, 46 and 100 ng TCDD/kg bw [148]. Cardio-myopathy and chronic active arteritis increased in a dose-dependent manner in TCDD-treated animals, with a NOAEL of 3 ng TCDD/kg and 46 ng TCDD/kg respectively. Cardiomyopathy was characterised by a slight increase in multiple foci of myocardial degeneration scattered within the ventricular walls. In most animals the severity of cardiomyopathy was graded as minimal [148].

Humans

The occupationally/accidentally exposed cohorts described in the cancer section have also been used in studies of TCDD exposure and cardio-vascular effects.

Cholesterol and triglycerides

A positive relationship between serum TCDD and total cholesterol concentration was found among Air Force personnel in Vietnam [149]. In a later follow-up study (Grubbs et al., 1995 in [10]) this relationship was less strong. In the same study group, TCDD concentrations were consistently associated with increased tri-glyceride concentrations [149](Grubbs et al., 1995 in [10]). British workers accidentally exposed to TCDD had higher cholesterol and triglyceride levels than controls that were not exposed in the accident [150]. No relationship was found between serum TCDD levels and the concentration of total cholesterol or high-density and low-density lipoproteins, either in industrial cohorts in USA and BASF-Germany [80, 151] or in Seveso residents [152, 153]. Similarly, no or very small differences in triglyceride concentrations were found in exposed subjects when compared to unexposed persons in these three cohorts.

Mortality

No increased mortality from diseases of the circulatory system in general or, more specifically, from ischemic heart disease was found in the German BASF cohort or in the Dutch occupationally exposed cohort compared to mortality in reference

populations [114, 117, 119]. In the Dutch cohort, mortality from ischemic heart disease increased with increasing estimated TCDD exposure within the cohort, whereas no such association was found in the BASF cohort [114, 117, 119].

In the German Boehringer cohort, an increased risk of mortality from ischemic heart disease with increased estimated TCDD and all PCDD/DF exposure was seen [154]. Workers in the US NIOSH cohort had an increased mortality from ishemic heart disease in comparison to the general US population [155], but no trend of increased mortality with increased estimated TCDD exposure was seen. When mortality was compared with an internal reference population, a trend of increased mortality with increased TCDD exposure was found [155].

No increase in circulatory disease mortality was found among Air Force veterans compared to a reference population of US Air Force veterans after 15 years of follow-up [156]. After 20 years of follow-up mortality in circulatory disease was still not increased in the Ranch Hand cohort compared to the reference population [157]. Among the enlisted ground crew veterans, however, mortality from circulatory diseases in general was significantly increased and, more specifically, from ischemic heart disease. Among a sub-group of veterans who had assayed serum TCDD levels, no trend of increased mortality with increased TCDD exposure was found [157].

The IARC International Cohort Study found no increase in circulatory system or ischemic heart disease mortality among workers in comparison to national mortality rates [140]. Among workers classified as exposed to TCDD or higher chlorinated dioxins, an increase in mortality was found from ischemic heart disease, but not from cerebrovascular disease [158].

In a study of mortality among New Zealand workers exposed to phenoxy herbicides and dioxins (n=1728), no increases in mortality from any of the studied circulatory diseases were found in comparison to national New Zealand mortality rates [130].

In a 20 year follow-up after the Seveso accident, no increase in mortality of circulatory diseases was seen among men and women living in the most heavily exposed zones A and B at the time of the accident, compared to mortality among residents in a reference zone [119].

Morbidity

A cross-sectional study of morbidity due to circulatory diseases was also performed on a subgroup within the NIOSH cohort, and no significant associations were found between TCDD exposure and cardiovascular outcomes

including myocardial infarction, angina, cardiac arrhythmias, hypertension, and abnormal peripheral arterial flow [159].

Conclusions

In conclusion, a few animal studies suggest that the heart is a sensitive target organ for TCDD effects, but the clinical significance of these effects is unknown. The human epidemiological studies show diverging results, and the sensitive endpoints studied in animals have not been addressed in humans. The human studies also have problems with exposure assessment and handling of possible confounding, so at this point in time no conclusion can be made about causality of associations between TCDD exposure and cardio-vascular disease mortality or morbidity.

Dermatological effects

Animals

Chloracne has been seen in TCDD-exposed cows and horses, on the ears of exposed rabbits and on the skin of exposed hairless mice. In the mouse the recessive 'hr-allele is required [143].

Humans

In humans, chloracne is the most widely recognized dermal effect of exposure to TCDD, and chloracne has been observed together with other effects in occupationally exposed individuals, but also in individuals where no other effects were observed [64].

Seveso

Among Seveso residents, chloracne was mostly found in children (88 % of cases) [160]. In the most highly exposed zone A, the prevalence of chloracne among children was 20 %. In 10 children with chloracne, levels of TCDD ranged from 820 to 56000 pg/g lipid. In 9 adults, without chloracne from the same area, serum levels ranged from 1770 to 10400 pg/g lipid [160]. In a follow-up of chloracne cases, about 20 years after the accident, health conditions of the chloracne cases were similar to those of controls from the Seveso area [161]. Chloracne risk was higher among residents younger than 8 years at the time of the accident and did not increase at puberty onset or during adolescence. A strong association was found between plasma TCDD levels and chloracne among subjects with light hair colour [161]. This indicates that genetic or environmental factors may modify TCDD toxicity.

Conclusions

A causal relationship between chloracne and TCDD exposure in humans is evident. The lowest TCDD concentration associated with chloracne in Seveso was 820 pg/g lipid, which shows that chloracne is a high dose effect. Data on exposure in epidemiological studies are uncertain since the contribution of direct dermal exposure to the induction of chloracne in both occupationally- and accidentally-exposed individuals can not be estimated. In cases when chloracne has been induced at least partly by direct dermal exposure, the body burden associated with chloracne may have been underestimated.

Diabetes

Diabetes, which is the most common serious metabolic disease in humans, arises from a number of defects in the regulation of synthesis, secretion and action of insulin. Elevated blood glucose level is the characteristic of diabetes, but this effect can be caused by a number of problems with the glucose balance in the body. Type 1-diabetes is caused by selective autoimmune-mediated destruction of the insulin-producing β-cells of the pancreas, mainly during childhood. This causes a decreased glucose uptake by the cells, causing increased levels in the blood. In type 2 diabetes, which is more common than type 1, the β-cells do not respond normally to increases in blood glucose levels, hence causing ”insulin deficiency”. Resistance to insulin action in the body is another common feature of type 2-diabetes. Factors such as age, obesity and lack of physical activity are associated with an increased risk of diabetes.

Animals

Links between TCDD and type 2 diabetes are lacking in experimental studies on animals, but mechanistic studies have indicated effects of TCDD on the expression of genes implicated in the type 2 diabetes disease [162, 163]. Lethal poisoning by TCDD involves reduced feed intake and disruption of energy metabolism (”wasting syndrome”) [164]. High acute exposures to TCDD cause reduced glucose uptake in different organs of guinea pigs, mice and rats [165] and lowers the insulin production by the pancreas in rabbits [166].

Humans

Possible associations between TCDD exposure and diabetes have been studied in a few of the occupationally-/accidentally-exposed cohorts [64]. The Ranch Hand cohort has been most extensively studied in this aspect.

Ranch Hand

In the Ranch Hand cohort, higher mean blood glucose levels were found in TCDD-exposed than in unexposed individuals [167]. The prevalence of diabetes mellitus (type not specified) and the use of oral diabetes medication increased with increasing TCDD exposure [167]. The time to onset of diabetes decreased with increased exposure to TCDD, and serum insulin abnormalities increased with increased TCDD exposure in non-diabetics [167]. Moreover, insulin levels were significantly increased in non-diabetic Ranch Hand veterans in the highest exposure category compared to the comparison group of other Air Force veterans who served in South East Asia during the same period, but who were not involved in Agent Orange spraying [157]. Significant interactions between sex hormone-binding globulin levels and insulin were found. There was, however, no difference in fasting glucose levels between comparison and Ranch Hand veterans [157].

In a study of US Air Force Veterans with background TCDD blood levels ($\leq$10 pg/g lipid), the risk of diabetes mellitus (type 2) was increased in the quartile of veterans with the highest TCDD levels compared to those with the lowest levels after adjustment for important risk factors for diabetes [168].

The study also looked at whether individuals with slow TCDD elimination who retain TCDD longer than individuals with faster elimination would have an increased risk for diabetes [169]. No association was found between elimination and diabetes, with or without adjustment for risk factors. After adjustment of covariates, no associations were found between elimination rates and time to onset of diabetes [169].

A comparison of insulin sensitivity in non-diabetic Ranch Hand veterans with high TCDD exposure with matched veterans from a comparison group did not show any significant differences [170]. A significant decrease in insulin sensitivity was, however, found with increased TCDD blood levels. The effect was small and it was concluded that the biological meaning of these results is difficult to resolve [170].

Industrial cohorts

In two industrial cohorts, higher mean blood glucose levels were found in TCDD-exposed than in unexposed individuals [80, 81].

Among trichlorophenol workers from two US chemical plants, the prevalence of type 2 diabetes was not different from the prevalence found in reference persons who had not been occupationally exposed to TCDD, and were living in the same area as the workers [81]. Moreover, prevalence was not associated with increased dioxin serum levels. In the US NIOSH study, diabetes mortality (any mention of

diabetes on the death certificate) was not increased in the exposed cohort [155]. Moreover, diabetes mortality was negatively associated with exposure within the cohort [155].

Seveso

In Seveso, the 20-year follow up after the accident found no overall increase in diabetes mellitus mortality in zone A and B compared to the reference zone R [119]. For females an increased risk was observed in zone A and B if only cases from the 15- and 20-year follow-up were included. No such excess risk was seen among men [119]. When mortality for all three zones were compared with the rest of the population in the two health districts of Seveso, total diabetes mortality for the entire follow-up period was significantly increased among women in the A+B zones, but not in the R zone [171].

Superfund cohort

A study of individuals (N=69, 2-94 pg TCDD/g lipid) with normal glucose levels during glucose tolerance testing and living near a Superfund site indicated hyperinsulinemia among the 7 individuals with the highest TCDD levels (>15 pg/g lipid) [172].

Background exposure

In populations with background exposure to organochlorines, mainly from food, correlations between lipid levels of highly chlorinated PCBs and PCB/PCDD/DF TEQs are high [47, 48, 173]. Consequently, PCB levels in lipids could be regarded as proxy markers of levels of TEQ. In a population-based study of 257 men and women, diabetic persons (type 2, 9 persons) had significantly higher serum levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs [174]. Moreover, a study of pregnant women showed that lipid-adjusted PCB levels were higher among diabetic women (mostly type 1) than in a non-diabetic control group [175]. In a study of 196 men and 184 women from a cohort of fishermen and their wives, lipid levels of the highly chlorinated PCB congener CB 153 and the DDT metabolite *p,p´*-DDE were positively associated with type 2-diabetes prevalence [176]. In a group of 205 elderly Swedish women, a positive association was found between HCB levels in serum lipids and diabetes mellitus (type not specified) [177].

Conclusions

Neither animal data nor human epidemiological data can be used in derivation of a TDI. Animal studies have focussed on mechanistic studies at high exposure levels, and links between TCDD and type 2-diabetes in experimental studies on animals are lacking. In the studies of highly exposed human cohorts, the results

are inconsistent. Moreover, in some studies diabetes mortality has been reported, although it should not be expected that diabetes (especially type-2 diabetes) is the cause of death. The most probable scenario is that diabetes may have contributed to cardio-vascular death. Interpretation of the diabetes mortality results is therefore difficult. In most human studies, exposure assessments are uncertain since TCDD levels in body lipids have often only been measured in sub-groups of the cohorts many years after the exposure has ceased. It cannot be excluded that diabetes might cause elevated blood levels of TCDD and other persistent and lipid-soluble organochlorines. Patients with type 2-diabetes are often advised to lose weight, and weight loss causes increased blood levels of organochlorines [177, 178]. Moreover, it cannot be excluded that the disease causes changes in lipid metabolism and distribution, thus indirectly influencing the distribution of organo-chlorines in the body. Finally, diabetic individuals may have lived a more ”organochlorine-dirty” life, due to high intake of fat-rich foods of animal origin, which contain higher organochlorine levels than low-fat animal foods and vegetables/fruits.

Table 5. Immunotoxic effects of TCDD in animals.

Species	Dose ng/kg/d NOEL	LOEL		Effect	Ref
Mouse	50	100	Single ip	PFC response SRBC	[179]
	100	1000	Single oral	PFC response SRBC	[180]
	300	1000	Single oral	PFC response SRBC	[181]
Mouse	5	10	Single oral	Mortality influenza	[182]
		100	Single ip	Mortality influenza	[183]
	500		Single oral	Mortality influenza	[184]
	100	1000	Single ip	Mortality influenza	[185]
Marmoset	3		Single sc	Changes in lymphocyte	[186]
Mouse		100	Single ip	subsets	[187]
Mouse	0.3		Weekly ip 135 d	Altered surface markers T-cells IL-1 mRNA	[188]

NOEL=No observed effect level; LOEL=Lowest effect level; ip= intraperitoneal; sc=subcutane; PFC=plaque forming cell response; SRBC=sheep red blood cells

Immune system

Animals

The immune system is an important target for toxicity of TCDD and other dioxin-like substances, causing suppression of both cell-mediated and humoral immunity in animals, with large species differences in sensitivity [143]. Thymic atrophy is a common observation after acute TCDD exposures over 1 μg/kg body weight. At lower doses TCDD may affect the cell-mediated immune response by influencing T-cell function in several ways, including decreased delayed-type hypersensitivity (DTH) response, inhibited generation of cytotoxic T-lymphocytes and decreased in vitro proliferative responses of T-lymphocytes to mitogens and specific antigens [143]. Effects on humoral immunity, which operates through antibody producing cells, also includes immunosuppression, as indicated by TCDD-induced suppression of primary and secondary antibody response to sheep red blood cells (SRBC) and tetanus toxin in rodents [143]. The most sensitive end-points for dioxin effects are given in Table 5.

Species differences

There are, however, species differences in immune effects of TCDD. For instance, in mice suppression of primary antibody response to SRBC occurs at single doses below 1 μg TCDD/ kg (Table 5) [179-181]. In rats, high toxic doses of TCDD leads to an enhancement of this immune response [189]. Moreover, differences in immune response between different strains of rats and mice are evident. In C57Bl/6 and C3H/HeN mice, a significant inhibition of plaque-forming response to SRBC was seen a single ip dose of 1.2 μg/kg bw, whereas a dose of at least 6 μg/kg was needed for a suppression in DBA/2 and AKR mice [190].

One of the most sensitive effects reported in mice on the cell-mediated immunity is inhibition of cytotoxic T-lymphocyte generation after TCDD exposure. In rats, cytotoxic T-cell activity is, however, relatively insensitive to TCDD, even less sensitive than thymic atrophy [191]. Clark et al.[192] treated male C57Bl/6 mice with single weekly ip doses of TCDD for four weeks. It was indicated that generation of allospecific cytotoxic T cells, in lymph nodes stimulated *in vitro* with alloantigen, was decreased at a total TCDD dose as low as 4 ng/kg. The results are, however, difficult to interpret since no statistical analysis of the results was performed. Mechanistic *in vitro* studies indicated that the suppression of cytotoxic T cells was caused by induction of T-suppressor cells in the thymus, acting selectively against the cytotoxic T cell response [193]. The number of T-suppressor cells in splenocytes of $B6C3F^1$ mice was, however, not increased after oral exposure to 1 μg TCDD/kg for five days, as shown by the lack of *in vitro* suppression of humoral responses to SRBC and DNP-Ficoll antigen when T-cells from TCDD-treated mice were titrated into naïve splenocyte cultures [194]. The suppressive effects on cytotoxic T cell response, as indicated by Clarke et al.

[192], could not replicated after ip exposure of C57BL/6J mice to 3 μg TCDD/kg bw/week for four weeks [189].

Effects on lymphocyte subsets and biochemical markers

Other T-cell types are, however, sensitive to TCDD exposure in rats. Dietary TCDD exposure of male Leeds Wistar rats to approximately 17 ng/kg/day for 180 days (total dose 3 μg TCDD/kg b.w.) caused suppression of concanavalin A-induced *in vitro* expression of IL-2 receptors of splenic T-cells [195]. Likewise, chronic oral exposure of female C57B1/6 to approximately 30 ng TCDD/kg/day for 14-15 months caused subtle changes in the proportion of thymic $CD4^-CD8^-$ cells (increases) and $\gamma\delta^+$ thymocytes (increased) [196]. In female C57BL/6 mice, exposure to initial doses of 1, 10, and 100 ng/kg, and weekly maintenance doses of 0.2-20 ng/kg for 135 days did not cause dose-dependent changes in patterns of thymocyte sub-populations. mRNA expression of the cytokine IL-1β was, however, increased in liver, lung and thymus, with a NOEL of 0.34 ng/kg/d [188]. This exposure results in similar body burdens of TCDD as a single dose of 10 ng/kg [143]. Female C57BL/6 mice, exposed to a single ip dose of 100-50 000 ng TCDD/kg, showed an altered density of expression of surface marker molecules on thymocytes at 100 ng TCDD/kg and higher, whereas the percentage of cells was not affected (Table 5) [187].

Subtle changes in T-cell expression of surface antigens have also been found in marmoset monkeys (Table 5). Animals exposed to single subcutaneous doses of TCDD exhibited a decreased percentage of peripheral T-cells and expression the surface antigen $CD4^+$ 4 weeks after exposure to 10 ng TCDD/kg bw. Within the $CD4^+$ population, significant decreases of ”helper inducer” or ”memory” cells were found. The total number of $CD4^+$ cells was, however, not affected [186]. An increased percentage of NK-cells and a decreased percentage and number of B-cells ($CD20^+$) was also indicated at 10 ng/kg bw. *In vitro* stimulation of peripheral lymphocytes with poke-weed mitogen resulted in a lower percentage of $CD4^+$ cells at exposures to 10 ng/kg and higher, whereas the percentage of $CD8^+$ cells was higher at these exposure levels [186]. NOEL in these studies was 3 ng TCDD/kg bw. In marmosets exposed to 0.3 ng TCDD/kg bw per week for 24 weeks, the effect on ”helper inducer” cells was opposite to the effects seen in the single dose experiments, i.e. the percentage increased in TCDD-dosed animals [197]. When the dose was increased to 1.5 ng/kg bw/week after 24 weeks the increase was reversed and a decrease was seen [197]. No functional deficit of the immune system could, however, be associated with the altered lymphocyte subset profiles [198].

In male marmoset monkeys exposed to a single subcutaneous injection of 100 ng/kg up- and down-regulation of thymic proteins were seen 4 weeks after exposure [199].

In female Rhesus monkeys fed 25 ppt TCDD in the diet (approx. 0.8 ng TCDD/kg/day) for 4 years, it was indicated that the percent of cytotoxic/suppressor T cells and helper/inducer T cells, respectively, was increased and decreased [200]. This led to a lower CD4/CD8 ratio in TCDD-treated animals than in controls. As stated by the authors, the percentages in TCDD-exposed animals were within the normal range, and no clinical evidence of an immune deficit was seen in TCDD-exposed animals. The reproduction capacity of the 25 ppt monkeys was, however, severely affected. Later studies on these monkeys have shown elevated serum concentrations of 1,2,3,6,7,8-hexachlorodibenzofuran and a tetra- and a penta-chlorinated PCB in TCDD-exposed animals, which makes it difficult to assess if observed effects were due to TCDD or not [16].

Groups of 5-6 female cynomolgus monkeys were orally exposed to 1-25 ng TCDD/kg/day in gelatin capsules 5 days/week for 12 months after surgical auto-implantation of endometrial strips at multiple abdominal sites [201]. After 12 months of exposure, the serum concentrations of interleukin (IL)-6 were decreased, whereas levels of soluble IL-6 receptors were increased at the highest dose [201].

Primary antibody production

In B6C3F1 female mice exposed to single ip doses of 0, 5, 10, 25, 50, 100, 500, 1000 and 5000 ng/kg, a NOEL of 50 ng/kg was found when the splentic antibody plaque-forming cell response (PCF) to SRBC was determined 9 days after TCDD dosing (Table 5) [179]. There were, however, several uncertainties in the experimental design and the statistical analysis of the results, making this study difficult to use in risk assessment. No information about the number of animals in the experiment was found in the publication. In the other experiments in the study only 3 animals per group were used. The statistical analysis was not performed with the control group as the reference group. Instead, the lowest TCDD-exposed group was used (5 ng/kg bw). Finally, TCDD was given as a single ip dose, which is not ideal if risk assessment of oral exposure is to be performed.

The next most sensitive study we found was a study on B6C3F1 female mice (14 animals/group) exposed to a single oral dose of 0, 0.1, 0.3, 0.5, 1.0 and 3.0 µg TCDD/kg (Table 5). One week after exposure to TCDD, all mice were immunised with SRBCs. Four days after immunisation, the PFC assay was performed. A NOAEL of 100 ng/kg bw was found when PFC was measured by serum anti-SRBC IgM [180]. In a recent study PFC response to SRBC was determined in C57BL/6N female mice (6-8 animals/group). The animals were dosed with a single oral dose of 0, 0.03, 0.1, 0.3, 1.0, 3.0 or 10.0 µg TCDD/kg bw. The same experimental protocol was used as in the previous study [180], with the exception

of the timing of the PFC assay occurring five days after the SRBC immunisation [181]. A NOAEL of 300 ng/kg bw was found.

Host resistance

Several studies have reported effects of TCDD on host resistance to infectious organisms, such as bacteria, viruses and parasites. Male C57Bl/6 mice were, at the age of 4 weeks, exposed to 0.5-20 µg TCDD/kg by gavage once a week for four weeks [202]. The mice were then challenged with *Salmonella bern* bacteria or *Herpesvirus suis* two days after the last TCDD dose. Increased mortality in response to *S. bern* bacteria was seen at the 1 µg TCDD/kg dose, and 5 µg TCDD/kg caused a shorter latency period before death. Swiss Webster female mice fed with feed containing 10-100 ng/kg TCDD for 8 weeks showed a compromised resistance to Salmonella infection [203].

The most sensitive infection study was a study on female B6C3F1 mice, where an increased mortality after intranasal infection with influenza A/Hong Kong/8/68 (H3N2) virus was seen after single oral doses of 10, 50 or 100 ng/kg bw (Table 5). NOAEL for mortality was 5 ng/kg bw after 22 days of observation [182]. The virus dose was adjusted to produce a 30 % mortality in the controls, and infection occurred 7 days after TCDD exposure. Mortality among TCDD-exposed animals was not associated with increased viral load [182]. In another study, female B6C3F1 mice (age 6-8 wk) were exposed to 0, 0.1, 1.0 and 10.0 µg TCDD/kg by single ip injection. After 7-10 days, mice (20/group) were infected intranasally with Influenza A/Taiwan/1/64 (H2N2) at a titre producing 10-30 % mortality [183]. Mortality was significantly increased in all TCDD-dosed groups, compared to the infected control, with a LOAEL of 0.1 µg TCDD/kg after 23 days of observation. In the same study mice (20/group) were also challenged with the bacterium *Listeria monocytogenes*, at a levels causing 20 % mortality. In this case, no mortality occurred in the TCDD-exposed animals [183].

A few studies have tried to repeat the results of the two initial studies, but no study has observed increased mortality at such low single doses as 10-100 ng TCDD/kg bw. For instance, four strains of mice (B6C3F1, BALB/c, C57BL/6N, DBA2), both females and males (10 animals/group), were exposed to a single oral dose of 0, 5, 20, 100 or 500 ng TCDD/kg 7 days before viral infection [184]. Animals were infected intranasally with influenza virus A/PR/34/8 (H1N1) at a level inducing approximately 30 % mortality in controls [184]. TCDD doses up to 500 ng/kg did not increase mortality among infected mice of any strain or sex during the 20-day observation period. In another similar study, female B6C3F1 mice were exposed to a single dose of 0, 1, 10, 100 or 1000 ng/kg bw ip 7 days before infection to influenza A/Hong Kong/8/68 (H3N2) [185]. The intranasal virus dose was set at doses causing 10-20 % mortality among controls. No TCDD dose-related alterations in mortality were seen [185]. In a second experiment with

similar design, B6C3F1 female mice were exposed to a single ip dose of 0, 25, 500 or 10 000 ng TCDD/kg bw, and in this experiment a NOAEL of 500 ng/kg bw was found [185].

Several other studies have used different experimental protocols, with different time-spans between TCDD dosing and influenza infection, and using different titres of influenza virus. Female B6C3F1 mice (age 7-9 wk, 6-10 animals/group) were exposed to a single oral dose of 1, 5 or 10 μg TCDD/kg one day prior to intranasal infection to influenza virus (H3N2) at a level that typically does not cause mortality [204]. Several similar experiments were performed, and the mortality results had substantial variation. For instance, in 2 out of 8 experiments no mortality was observed in mice exposed to 10 μg/kg bw, whereas in one experiment 80 % of the animals died [204]. In another study, a NOAEL of 1 μg TCDD/kg (single oral) was found for mortality in virus-infected female B6C3F1 mice after an H3N2 infection (24 h after TCDD exposure) causing no mortality in the infected controls [205].

Humans

In the WHO risk assessment from 2000, studies on cohorts exposed to TCDD in industrial settings, through accidents and during the Vietnam war were reviewed [64]. In Seveso, small and transient changes in lymphocyte counts were seen in TCDD-exposed patients shortly after the accident 1976 [206]. Immunocompetence was not affected when tested in 44 children from the most contaminated A zone in the Seveso cohort [207], and the results were within the normal range. A slight increase in proliferative response of lymphocytes to mitogens was observed in the exposed children when tested in 1985 [206]. Studies of immune system functions of occupationally or accidentally exposed cohorts from the US, Germany and Great Britain have shown slight alterations in some lymphocyte subpopulations, and in immunoglobulin, immune complex and complement levels in blood [80, 208-210]. In the cohort including Vietnam war veterans exposed to TCDD during the handling and use of the herbicide Agent Orange (Ranch Hand cohort), no evidence of a consistent relation between back-extrapolated blood TCDD levels (background-3300 pg/g lipid) and immune system alterations was found 20 years after the end of the Vietnam war [210].

Further studies of TCDD-exposed German chemical workers found changes in the proportions of certain lymphocyte sub-populations and interferon γ release from whole blood cultures [211, 212]. No associations were, however, found between PCDD/DF levels in blood and occurrence of infections during and after the occupational exposure to dioxins [212]. No association was found between PCDD/DF levels and concentration of tetanus antibodies three weeks after vaccination or in the outcome of the chromate resistance test. It was noted that the

cohort, apart from PCDD/DF exposure, also had been exposed to other chemicals such as β-HCH and benzene [212].

In other studies of German chemical workers, no clear-cut changes were found in percentages of lymphocyte subpopulations compared to a reference group [213]. From this study the authors concluded that humans are much less susceptible to dioxin-related effects on helper-inducer T cells than marmoset monkeys [213]. In a further study of *in vitro* proliferation of lymphocytes from the same study participants, no suppressive effects were seen on proliferations after in vitro exposure to several mitogens/antigens [214]. Finally, in an extended study population including more dioxin-exposed workers, a statistically significant decrease in blood IgG1 levels was found in workers exposed to TCDD, but not in those exposed to PCDD/DF [215]. There were no dioxin-related changes in any of the other immunoglobulins studied or on cytokines and TNFα.

In a study of 62 randomly selected individuals from the A and B zones and 58 controls from the surrounding area, plasma IgG levels decreased with increasing TCDD plasma concentration after adjustment of possible confounders [216]. IgM, IgA, complement 3 and complement 4 did not exhibit any consistent association with TCDD levels.

Immune status (leukocyte counts, lymphocyte subpopulations, immunoglobulins, complement, and in vitro antigen stimulation of lymphocyte proliferation) was determined in 259 male workers from two US plants producing 2,4,5-trichlorophenate, and in a control group of 243 unexposed referents who lived in the communities of the workers [217]. Serum TCDD concentrations (background-3400 pg/g lipid) were negatively associated with circulating CD26 cells and spontaneous background proliferation of lymphocytes. In linear regression analysis, only 1-2 % of the variation in the immunological outcomes was explained by the variation in TCDD levels [217].

There are some epidemiological studies of effects of dioxins on immune function in populations with background exposure, but the results are difficult to interpret because of co-exposure to non-dioxin-like PCBs and other organochlorines. Dutch studies have found significant associations between post-natal PCB exposure and increases in prevalence of certain diseases, such as middle ear infections during child-hood [218, 219]. In a study of adolescents from Belgium, percentages of eosinophils and natural killer cells in blood were negatively correlated to dioxin-like TEQ levels in serum [220]. Levels of IgA and specific IgE were negatively associated to TEQ levels. Studies of men with high consumption of PCB- and dioxin-contaminated fatty fish from the Baltic Sea have found significant associations between fish consumption or blood levels of certain PCB congeners and changes in proportions of lymphocyte subsets [221, 222].

Conclusions

Immunotoxicity is a sensitive effect of TCDD in animals, and associations between TCDD exposure and effects on the immune system have been observed in highly exposed human populations. However, epidemiological results are inconsistent and conclusions about causality cannot be drawn. Confounding problems have rarely been addressed. Moreover, clinical consequences of possible effects on markers of immune function in humans still have to be elucidated. None of the epidemiological studies of occupationally or accidentally exposed cohorts have indicated severe immunosuppression among highly exposed sub-groups.

In animals, four TCDD-sensitive endpoints were identified: increased expression of IL-1 mRNA in organs of mice, alterations in proportions and number of lymphocyte subsets in marmoset monkeys, increased mortality after influenza infection in mice and suppressed primary antibody response to sheep red blood cells in mice (Table 5). Expression of IL-1 mRNA and alterations in lymphocyte subsets have not been linked to an increased risk of disease, and could be regarded as early markers of effects that may or may not lead to adverse effects.

The most sensitive adverse immunological effect in animals is increased mortality in influenza-infected mice exposed to TCDD (NOAEL: 5 ng/kg bw, single oral dose) [182]. A few studies have tried to repeat this effect at low exposure levels, but the efforts have failed [184, 185]. Moreover, other studies using slightly different experimental protocols have not been able to induce increased mortality rates at low TCDD exposures [204, 205, 223]. Due to the inconsistence and the uncertainties about the mechanisms behind this large variability in response to TCDD [205, 223-225], these studies were not used in the calculation of TDI.

The PFC response after immunisation with SRBC is also a sensitive adverse effect of TCDD in animals, with NOAEL in three mice studies ranging from 50-300 ng/kg, single dose. Many uncertainties were, however, found regarding the interpretation of the results in the study with the lowest NOAEL [179]. The next lowest NOAEL found by us was 100 ng/kg bw after a single oral TCDD dose [180]. This study was of better quality, with a high enough number of animals (14) in each group and with a good description of the statistical analysis of the results.

Teeth and bones

Effects on teeth

Alteration in tooth development was recognized as a sensitive effect of dioxin in the WHO risk assessment from 1998 based on studies on rats and mice. Since then, tooth development has also been identified as one of the most sensitive effects of dioxin exposure in monkeys and humans. Several studies have confirmed this observation in experimental animals as well as in humans. These studies show that the developing tooth anlagen and the mineralisation process are sensitive to dioxin. Early exposure can result in missing teeth or reduced size, whereas later exposure can lead to mineralisation defects. In humans, the mineralisation continues for a long period after birth, i.e. up to the age of around 20. Thus, although a developmental effect, it can be dependent on direct exposure of the child or teenager and not only on indirect exposure via the mother.

Animals

Studies in rats have shown that exposure to TCDD on GD15 decreases the number of 3rd molars at dose levels from 0.3 µg/kg (LOAEL) [226]. The size of the 3rd molar was decreased at doses of 0.03 µg/kg and above. The most sensitive stages are the early bud stage and the mineralisation stage [227]. Depending on which tooth, the sensitive windows occur either pre- or postnatally. Colour defects, pulpal perforation and root formation defects have also been reported to be caused by TCDD [228, 229].

Humans

In a Finnish study on breast-fed infants from the general population, both the frequency and severity of hypomineralisation lesions at age 6-7 years correlated with total PCDD/F exposure during lactation [230]. In another study, the number of tooth defects has been correlated to the length of lactation, although dioxin levels were not measured in this study [231]. In higher exposed Yu-Cheng children, tooth defects have been dose-dependently related to exposure to PCBs/PCDFs [232]. In Seveso, the incidence of developmental enamel defects and hypodontia correlated to serum TCDD levels for the children who were less than 5 years of age at the time of the accident [233]. In contrast, other dental disorders, such as dental caries and periodontal disease, oral pigmentation or salivary flow rate were not related to TCDD-exposure.

Although not studied in relation to dioxin exposure, a Swedish study has shown that children born in 1970 had more idiopathic enamel hypomineralisation than those born before and after [234]. This period coincides with the period when the highest levels of dioxin were measured in Swedish mother's milk [235].

Effects on bone

In recent years, dioxins and dioxin-like PCBs have been reported to cause effects on bone in both experimental animals and in humans. These effects were not described in the risk assessments of WHO or EC/SCF.

Animals

Several studies show effects of PCB (also the dioxin-like PCB 126) on bone in different experimental and non-domestic animal species. Studies of TCDD and PCB 126 in rodents are described below. Lind *et al*. [236, 237] reported that PCB 126 impairs bone strength and alters bone composition and size in SD rats. The effect of PCB 126 on bone was modulated by estrogen [236, 238]. In a tumour promoter study with exposure to TCDD weekly for 20 weeks, female L-E rats showed inhibited tibial growth (both length and area) [239]. Bending and breaking force and stiffness were also reduced. L-E rats were more sensitive to these effects than H/W rats, suggesting the involvement of the AhR in these effects. In the sensitive L-E rats these effects occurred at a total dose of 1.7 µg/kg, but not at 0.17 µg/kg (NOAEL). The NOAEL dose corresponded to a final liver concentration of TCDD of 0.67 ng/g dry weight. In a study with *in utero* and lactational exposure of TCDD, similar effects of inhibited bone growth and reduced bone strength were reported [240]. Gestational exposure was the most sensitive window, although effects also occurred after only lactational exposure. A single dose of 1 µg/kg caused these effects, whereas 0.3 µg/kg did not (NOAEL). Levels of TCDD in the offspring at birth were approximately 25 pg/g fresh weight and 2500 pg/g lipid. The concentration in the dam was somewhat lower, approximately 2200 pg/g lipid.

Humans

In a study on organochlorines and bone mineral density in Swedish men from the general population, no relationships between bone quality and levels of any of the compounds analysed, including PCBs, could be observed [241]. Later studies on the Fishermen cohort from the Swedish east coast gives some support to the assertion that consumption of fatty fish from the Baltic Sea (polluted with dioxins, PCBs and other organochlorines) is a risk factor for osteoporotic fractures [242, 243].

Conclusions

To summarize, the effect on tooth development is sensitive and important in view of a high exposure to dioxins during breast-feeding, a period when many permanent teeth develop. Such effects could also occur in other teeth through exposure after breast-feeding. However, a high enough dioxin exposure to cause these effects is not probable in the general population. Regarding the effect on

bone, studies suggest that this effect is more sensitive when exposure occurs during early development. However, available data are not sufficient for assessing the sensitivity for this effect as a result of adult exposure. Neither the tooth nor the bone studies can presently be used for quantitative risk assessment due to lack of dose-response data.

Reproduction

Reproduction is one of the most sensitive systems to dioxin exposure. Here we describe the data available for assessing the risk for effects on male and female reproductive systems and fertility.

Male reproduction

Sperm production in animals

Several studies on TCDD and other dioxin-like compounds have reported adverse effects on male reproduction. However, such effects have often been shown at doses causing severe toxicity. More recently, studies in both young and adult rats have shown that sperm production is the most sensitive endpoint to TCDD in male reproduction [100, 244]. A single oral dose of 10 µg/kg causes decrease in both daily sperm production and cauda epididymal sperm reserve (NOAEL 3 µg/kg) [244]. Higher dose levels resulted in decreased body weight and serum testosterone levels. When pregnant dams were exposed at GD15 a dose level of 0.3 µg TCDD/kg was sufficient to reduce sperm production in male offspring (NOAEL 0.1 µg/kg) and 1 µg/kg caused a decreased anogentital distance (AGD) [100].

Sperm production in humans

Effects on sperm parameters have been reported for men prenatally exposed to PCBs and PCDFs during the Yu-Cheng accident [245]. Similar effects, such as higher abnormal morphology and oligospermia rate, as well as a lowered ability of sperm to penetrate hamster oocytes have also been reported for men who were exposed to the contaminated rice oil as adults [246]. Studies on the Swedish Fishermen cohort exposed to dioxins and other organochlorine pollutants via the fatty fish from the Baltic Sea have also focused on male reproduction. The results of these studies suggest that these pollutants may cause a slight negative impact on human sperm chromatin integrity and a decreased sperm motility, and may increase the proportion of ejaculated Y-bearing spermatozoa [247-249].

Sex ratio

In Seveso, the sex ratio of children born after the accident was completely changed [250]. In nine families where both the mother and father were highly exposed to TCDD, twelve girls and no boys were born in 1977-1984. Paternal blood concentration emerged as the most important predictor of a lowered sex ratio at birth [251]. A similar effect on the sex ratio was observed for men exposed to contaminated rice oil in Yu-Cheng [252]. The decreased sex ratio was, however, only observed for men exposed to TCDD at the age of <20 years. However, another study on highly exposed men could not observe such an effect. In a study on male workers exposed to high levels of TCDD (serum levels of 3-16340 pg/g TCDD) no effects on spontaneous abortions or on the sex ratio could be found [253].

Female reproduction

Animals

Several studies report that dioxins affect the female reproduction system. However, the most sensitive effect was observed in a long-term feeding study in Rhesus monkeys [14] showing a dose-dependent increase in the frequency of endometriosis in elderly females exposed to low dose levels of TCDD. An increase was observed already at the lowest dose level 5 ppt in the diet. These monkeys also showed a relatively high spontaneous frequency of endometriosis in the control group. This study was one of five critical studies used for setting the WHO TWI in 1998. In the risk assessment by EC/SCF in 2000 [10], the monkey study was among those considered critical for setting the TDI. The dose level at LOAEL was calculated to 0.15 ng/kg/d and the corresponding body burden at the end of the exposure period was 39 ng/kg bw. However, in 2001 the SCF [16] identified some problems in the study, e.g. the very high spontaneous incidence of endometriosis, uncertainties regarding the body weights of the monkeys (dose calculations), and the presence of other dioxins and PCBs in the monkeys [17]. The Committee decided not to include this study as a pivotal study, though it recognized that effects were reported at body burdens similar to those calculated for the critical effects in the rat studies. Recently, immunological parameters have been studied in the same Rhesus monkeys showing effects in the endometriosis cases [254]. However, the basic problems for interpretation of the study remain. Endometriosis does not occur in rodents, but TCDD has been shown to increase the growth of surgically-induced endometriosis in both rats and mice, although at much higher doses than in the monkey study [255, 256].

Humans

The menstrual cycle has been studied in women exposed to dioxins and dioxin-like compounds. Adolescent girls exposed prenatally to PCBs and PCDFs in the Yu-Cheng accident had a shorter duration of menstrual bleeding, a higher rate of

irregular menstrual cycles and higher serum levels of estradiol and FSH in the follicular phase of menstrual cycle, compared to a control group [257]. In a Swedish study, women from Fishermen families eating relatively large amounts of the polluted fish from the Baltic Sea showed shorter menstrual cycles than women from Fishermen families on the Swedish west coast [258].

A study of endometriosis in Seveso showed a doubled, but not statistically significantly increased, risk in women with serum levels of 100 ppt TCDD or higher [259].

Several case-control studies have been performed on non-accidentally exposed women. Some of these studies show higher levels of dioxins in endometriosis cases than in controls, while others find no difference between cases and controls. A recent Belgian study of women of reproductive age (50 cases and 21 controls) shows that total TEQ-levels are significantly higher in cases compared to controls [260].

Time to pregnancy (TTP)

TTP can be used as a tool for assessing reproductive success in humans. A long TTP can be due to decreased fertility in either the male or female, but can also have other explanations, such as bad luck. A series of studies on the Swedish Fisherman cohort has been performed in order to evaluate any effects on fertility, TTP and miscarriages [261-263]. No support for a negative effect of the consumption of fatty Baltic fish on the TTP, miscarriages or subfertility could be observed [262]. In one study, a decreased rather than an increased TTP was indicated [263]. The only negative effect on pregnancy rate was observed for heavy smokers from the East coast cohort [261].

Conclusions

To summarize, the effects described above regarding male and female reproduction may all be of relevance to the risk assessment of dioxins. However, the data available are not sufficient to base the risk assessment on. Further research is needed regarding causality (endometriosis, sex ratio) and threshold exposure for effects (all effects). In addition, the mixed exposures in the epidemiological studies (Yu-Cheng, Swedish fishermen) lead to difficulties in interpretation of the findings. However, if causal relationships exist, the studies indicate that at least endometriosis may be a very sensitive effect of exposure to dioxin.

Development of Tolerable Daily Intake (TDI)

Introduction

It is generally assumed that there is a receptor-based mechanism behind all important dioxin-related effects, including cancer. A receptor-based mechanism most probably leads to a dose-response curve with sigmoid shape. As a consequence, in practice a threshold approach can be used in risk assessment.

A TDI for non-genotoxic compounds, such as dioxins, is based on an estimate of the threshold dose for the critical effect(s), i.e. the most sensitive adverse effect(s) with relevance for human health. After defining this threshold, often a NOAEL dose, assessment factors are applied to cover different kinds of uncertainties, e.g. interspecies and inter-individual variation in sensitivity. As an example, the EC/SCF risk assessment of dioxins and dioxin-like PCBs based the TDI on the most sensitive developmental effects of TCDD in rats, which were decreased sperm production and altered sexual behaviour in male offspring after *in utero* exposure. An assessment factor of 3 was applied to account for the use of a LOAEL instead of a NOAEL, and a factor of 3.2 accounted for inter-individual variation in toxicokinetics within the human population [10, 16]. Additional factors may be applied to protect highly sensitive groups, to compensate for lack of data, or if the critical effect is regarded as very severe (e.g. cancer), as suggested by Falk-Filipsson et al. [264].

Critical endpoints for non-developmental effects of TCDD

The present risk assessment focusses on health risks of dioxin at non-developmental exposure. TCDD and other dioxin-like compounds are toxic to both animals and humans at high doses. In humans chloracne has consistently been observed in populations with high dioxin exposure. Many of the effects of TCDD studied in animals and humans, such as cardio-vascular effects, diabetes, effects on teeth and bone, and some reproductive effects, can currently not be used in the calculation of TDIs. It is not possible to draw firm conclusions about causality between exposure and effects in humans, as effects are not consistent in human studies, effects occur at high doses (animal studies), or because dose-response relationships are not known (see conclusions under each endpoint

above). The present evaluation of non-developmental toxicity of dioxins concludes that cancer and immune toxicity are the effects that at present can be used for calculating a TDI. However, further research may or may not identify more sensitive effects.

TCDD has been classified as a human carcinogen, based on sufficient evidence in animals and limited evidence in humans, but with mechanistic considerations taken into account [106]. There are uncertainties in the epidemiological studies, for instance regarding exposure assessment and the possibility of confounding. In animals, cancer has been identified as one of the critical (most sensitive) effects of TCDD during adult exposure. Several lifetime studies in rats and mice show that TCDD is a potent carcinogen and causes dose-related increases of tumours in several organs. As TCDD is considered to be a non-genotoxic, yet potent tumour-promoting compound, a threshold approach is used for extrapolation to lower exposure levels in humans. Hepatic tumours in female rats are the most sensitive neoplastic effect of TCDD. However, at higher dose levels tumours appear at multiple sites and also in males. At such dose levels a decrease occurs in hormone-related tumours in the uterus, mammary glands and pituitary gland.

Due to the marked species differences in the elimination half-life of TCDD, a body burden approach has been used for dose extrapolation from rats to humans in the most recent risk assessments [4, 10, 16]. The same approach for interspecies extrapolation, but based on lipid concentrations of TCDD, is used in the present report (see below).

Immunotoxicity is also a sensitive effect of TCDD in animals. Associations between TCDD exposure and effects on markers of immune function have also been observed in highly exposed human populations (see immunotoxicity section above). However, epidemiological results are inconsistent and conclusions about causality cannot be drawn. Moreover, clinical consequences of possible immune effects in humans still have to be elucidated. In animals, suppressed primary antibody response in mice was identified as the most sensitive end-point [179, 180].

For primary antibody response (PFC) to sheep RBC NOAEL in the study of best quality was 100 ng TCDD/kg bw after exposure of mice to a single oral dose [180]. To be able to compare this study with the cancer studies the concentration of TCDD in fat was calculated. The body burden of TCDD was estimated to be 80 ng/kg bw, assuming an oral absorption factor of 80 %. From this body burden the TCDD level in body lipids was estimated to 800 ng/kg lipid using a body fat composition of 10 % in mice. The estimated TCDD level in lipids (0.8 ng/g lipid) at the immunological NOAEL was higher than the measured TCDD level at NOAEL in the Kociba cancer study and within the range of NOAELs of lipid TCDD levels in the NTP cancer study (Table 4).

The TCDD levels were not analysed in the immunological study. A further complication is that the single oral doses given in the study probably cause a peak in the blood TCDD level. The above ”theoretically” estimated lipid TCDD level may therefore underestimate the real TCDD exposure of the target tissues. We conclude that the tissue TCDD levels at NOAEL in the immunological study most probably were within the upper range of the levels in the cancer studies. Since the levels of TCDD were not measured in the immunological study, we found it appropriate to use the cancer studies in development of the TDI. Moreover, the immunological results were not tabulated in the published report [180] and could thus not be used in benchmark modelling (see below). However, immunotoxicity is one of the most sensitive effects of TCDD in animals and further data at low doses are required in order to improve risk assessment of TCDD.

In conclusion, in the present risk assessment of non-developmental exposure to dioxins, cancer was used as the critical effect and the basis for calculation of TDI.

The Benchmark Dose Approach

The use of a NOAEL in setting standards for human exposure to non-genotoxic compounds has been criticized [265]. Shortcomings associated with this procedure for health risk assessment include the following:
* The NOAEL is limited to the doses tested experimentally
* The shape of the dose-response relationship is not considered in the determination of the NOAEL
* Experiments involving fewer animals tend to produce higher NOAELs
* The NOAEL does not provide an estimate of the potential risk, or effect, associated with the exposure of interest

The benchmark dose (BMD) method has been suggested as an alternative approach to be used in health risk assessment [265]. The method involves fitting a mathematical model to dose-response data and the BMD is defined as the dose causing a predetermined change in response, e.g. 5 %. The lower 95 % confidence limit of the BMD, i.e. the BMDL, has been proposed to replace the NOAEL for determination of guidelines values, i.e. assessment factors are applied to the BMDL instead of the NOAEL. The BMD method is illustrated in Fig 1, using malformation data observed in mice fetuses following maternal exposure to TCDD [266, 267].

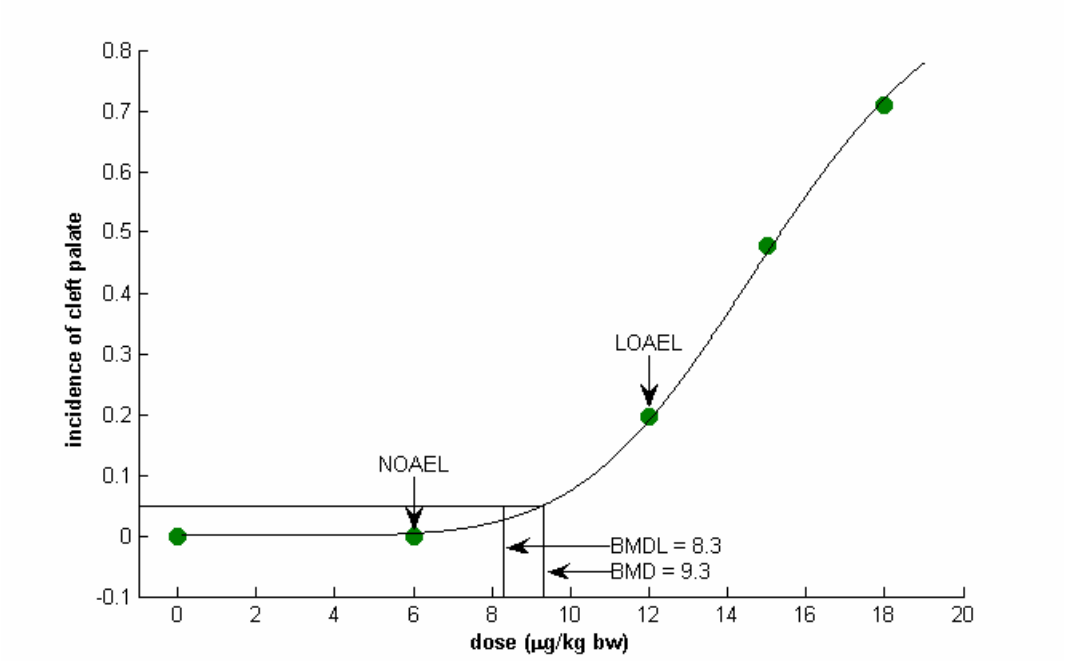

Figure 1. The BMD, and its lower bound (BMDL), defined as corresponding to an excess risk of 5 %. Incidence data on cleft palate observed in mice fetuses following maternal exposure to TCDD was used (data from [266]). The no-observed-adverse-effect level (NOAEL) and the lowest-observed-adverse-effect level (LOAEL) are 6 and 12 µg/kg bw, respectively.

A number of advantages of the BMD methodology compared to the traditional NOAEL procedure have been identified:

* The BMD method takes into account the shape of the dose-response relationship to a greater extent than the NOAEL approach and is not limited to the use of one experimental dose level.
* The use of the lower confidence bound (BMDL) appropriately reflects the sample size of a study, i.e. larger studies tend to result in shorter confidence intervals and thus lower uncertainty.
* A BMD, and a BMDL, can be calculated even on occasions when a NOAEL cannot be determined.
* The BMD, and BMDL, correspond to an explicit response level, which introduces consistency between studies and suggests that the human health risk assessment will be based on more scientific information in comparison to the NOAEL. Even though the NOAEL is a statistical ”no-effect level”, the response (relative to background) at the NOAEL can vary considerably between studies.

This last statement has been presented a major advantage, but it also represents one of the challenges associated with the benchmark methodology, i.e. which

level of response, or risk, should the BMD correspond to? For incidence data the BMD was originally presented as the dose corresponding to an additional, or extra, risk of 1-10 % (Crump, 1984). In the present risk assessment an extra risk of 5 % has been chosen (BMD05, BMDL05). Extra risk is interpreted as a percent increase in risk relative to the total response size, i.e. 100 % minus the background risk. If the background risk is zero, the additional (absolute increase over background) and extra risk definitions coincide. The 5 % level has most commonly been suggested in previous applications of the BMD approach [267-269].

Effects on tumour development have been identified as the most critical non-developmental effect of TCDD and there are sufficient data from the experimental studies for dose-response modelling. To be able to compare the carcinogenicity studies in rats [19, 108], despite the differences in experimental design, the most sensitive effects were modelled using BMD methodology. The resulting BMD and BMDL at the 5 % level, as well as corresponding NOAEL and LOAEL levels, are presented in Table 6 and Figure 2, 3 and 4.

Table 6. The most sensitive neoplastic effects of TCDD in female rats presented as BMD and BMDL at the 5 % level as well as NOAEL and LOAEL (ng TCDD/g fat at steady state).

Study	Endpoint	BMD05	BMDL05	NOAEL	LOAEL
Kociba 1978 [19]	Hepatic tumours (comb.)	0.83	0.59	0.54	1.70
NTP 2004 [108]	Hepatic tumours (adenoma)	2.41	2.01	2.00	3.18
	Liver cholangiocarcinoma	1.81	1.50	1.40	2.00
	Oral mucosa squamous cell carcinoma	2.24	1.56	2.00	3.18
	All organs, malignant tumours	1.35	0.66	1.40	2.00

Note: BMDs and BMDLs were estimated by the Weibull model using the USEPA BMD software (www.epa.gov/ncea). The log-logistic model was also considered in the analysis, but no appreciated model differences were observed. The Weibull model was selected since it provided slightly more conservative values.

Of the neoplastic effects from the cancer studies (Table 6) that showed the lowest BMDL levels, liver cholangiocarcinoma (Figure 2) in the NTP rat study [108] was used for the final calculation of TDI. Both hepatic tumours in the Kociba rat study [19] (Figure 3) and total malignant tumours of all organs in the NTP study [108] (Figure 4) resulted in slightly lower BMDL levels. For these endpoints the BMD05 and the BMDL05 were in the range of experimental doses where no effects were observed, i.e. at or below the NOAEL. Because of this the BMDL for liver cholangiocarcinoma was considered a more reliable starting point for the TDI calculation. Generally speaking, the NTP study is also preferable, relative to the Kociba study, in the BMD analysis (more animals per group and more dose levels studied in the NTP study).

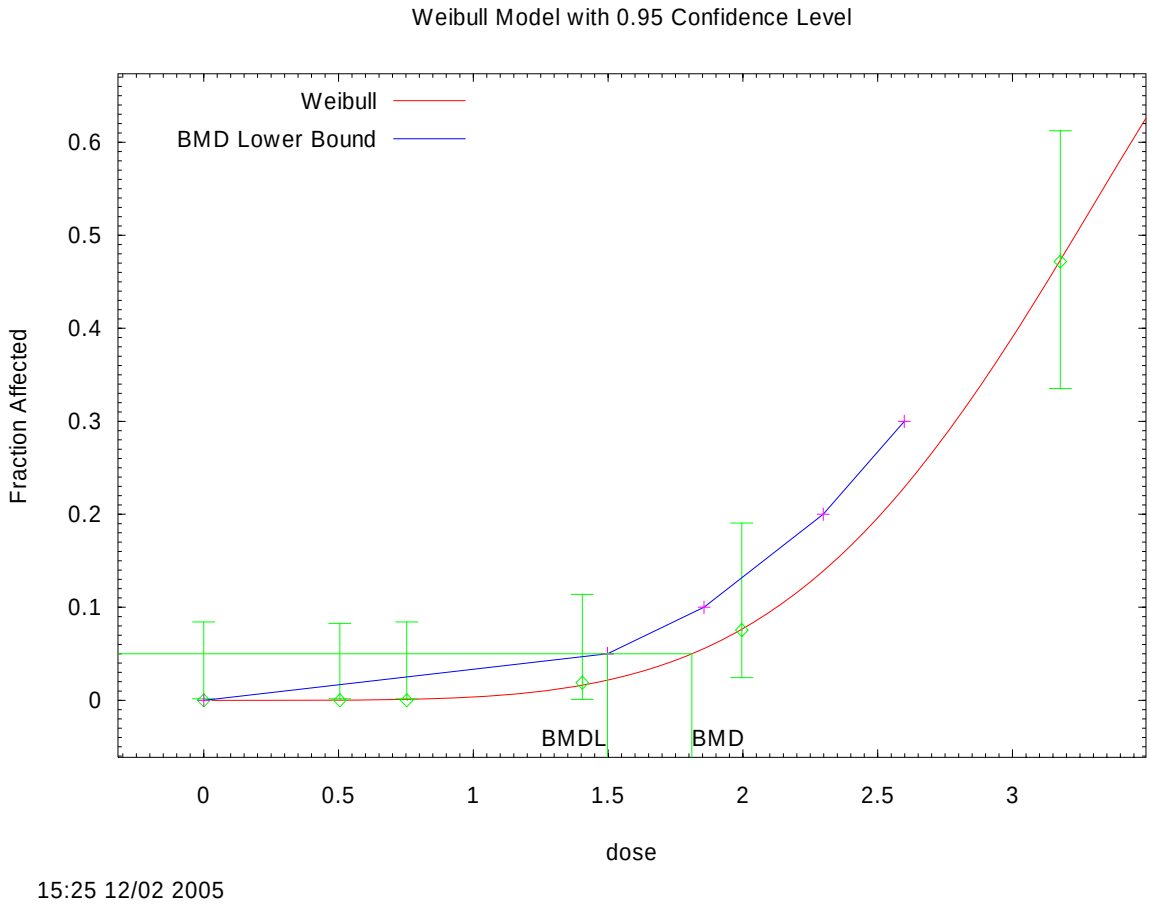

Figure 2. The Weibull model fitted to incidence data on liver cholangiocarcinoma from the NTP study [108]. The BMD and BMDL are 1.81 and 1.50 ng/g fat, respectively, and correspond to an extra risk of 5 %. Liver cholangiocarcinoma appeared to be the most sensitive neoplastic tumour from the NTP study [108].

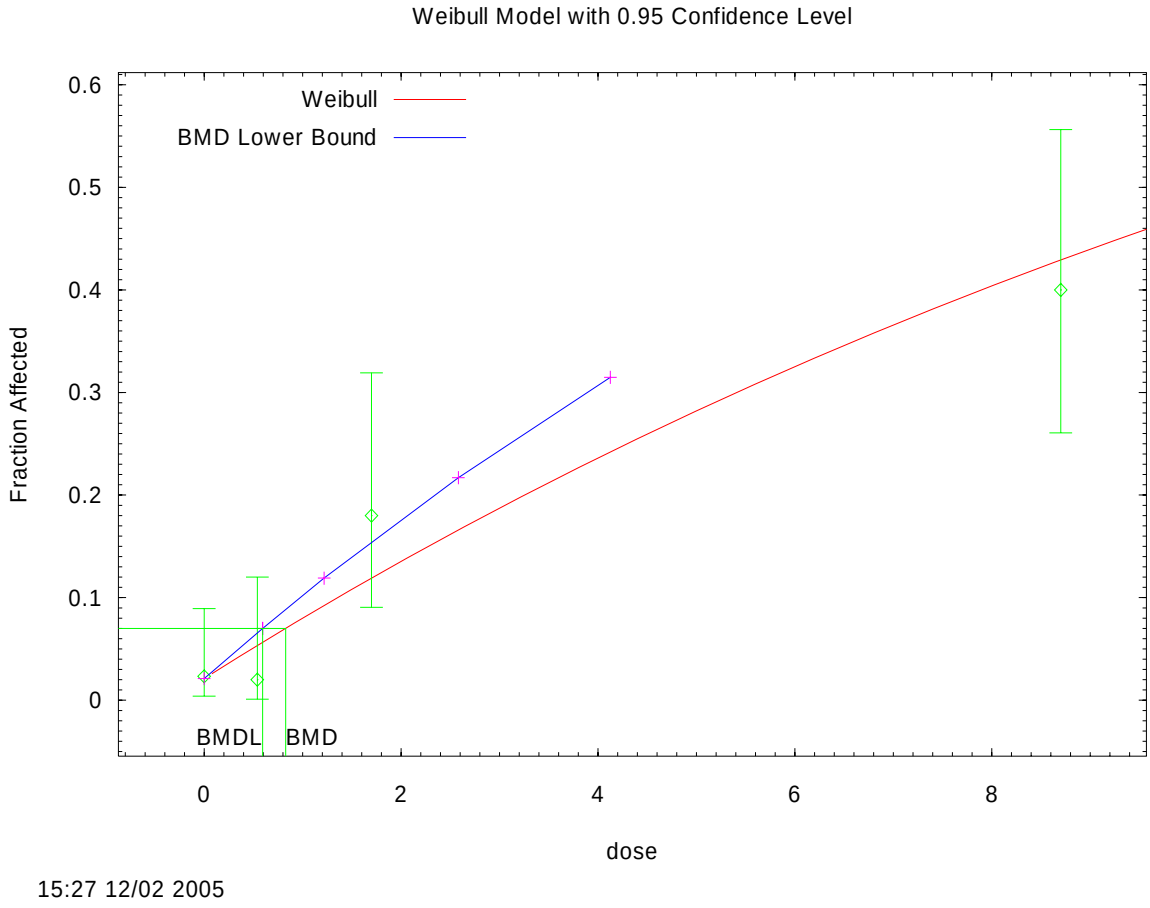

Figure 3. The Weibull model fitted to incidence data on hepatic tumours from the Kociba et al study [19]. The BMD and BMDL are 0.83 and 0.59 ng/g fat, respectively, and correspond to an extra risk of 5 %.

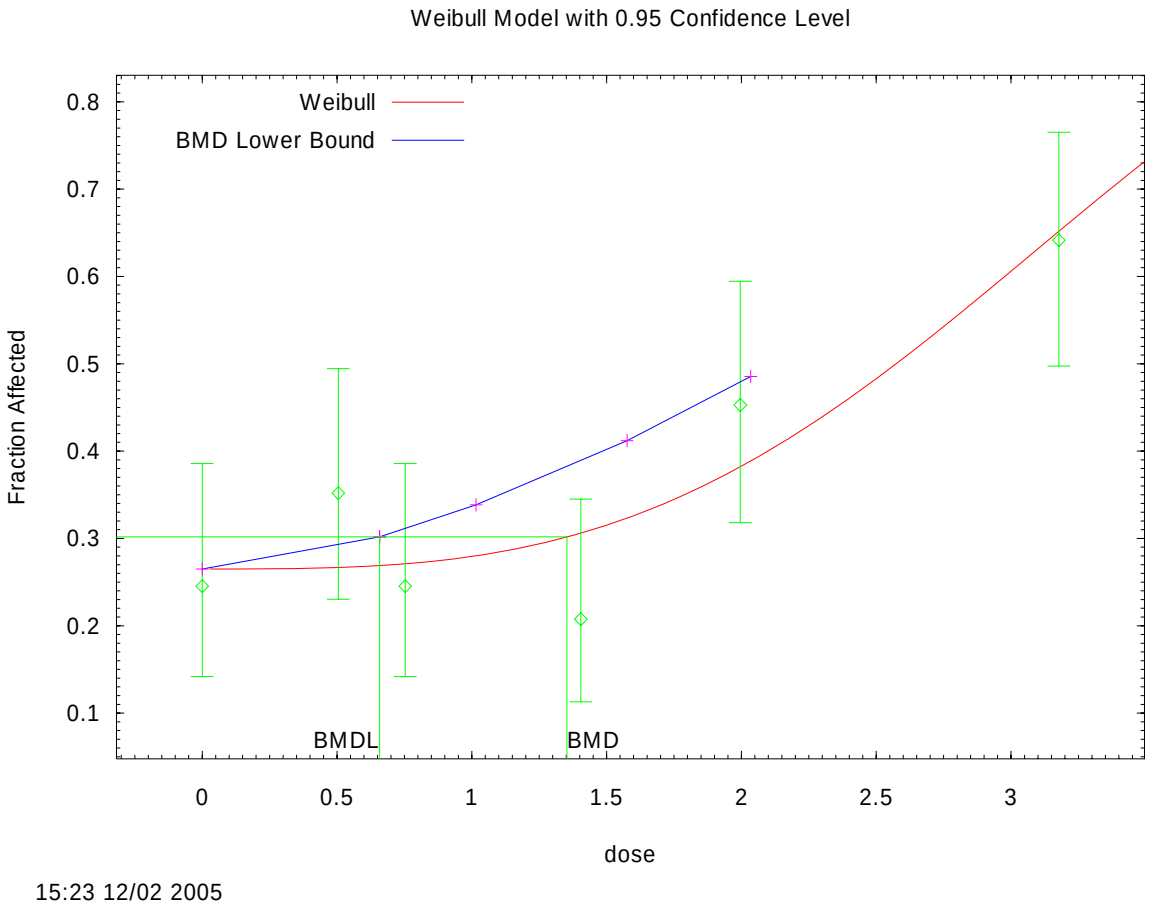

Figure 4. The Weibull model fitted to incidence data on all malignant tumours from the NTP study [108]. The BMD and BMDL are 1.35 and 0.66 ng/g fat, respectively, and correspond to an extra risk of 5 %.

Assessment factors

Introduction

In the 1950s it was proposed that an acceptable daily intake (ADI) of food additives or contaminants could be derived by dividing a chronic NOAEL from orally administered animals with a 100-fold safety factor. The ADI approach in risk assessment of food additives and pesticides is now widely accepted, as well as the corresponding tolerable daily intake (TDI) approach for contaminants. However, it is apparent that the choice of the value 100 was more or less arbitrary, with little scientific support. In attempts to increase the scientific transparency, the factor of 100 was split into the uncertainty factors of 10 for inter-species and

10 for inter-individual (human) variation [270, 271]. The main feature of this approach is that inter-species and inter-individual differences are distinguished in kinetic and dynamic (mechanistic) aspects. The International Programme on Chemical Safety [272, 273] has adopted these principles and suggested that the uncertainty factor for inter-species extrapolation is subdivided 4- and 2.5-fold respectively, for kinetic and dynamic differences (Figure 5). The uncertainty factor for inter-individual (human-to-human) extrapolation was suggested to be sub-divided evenly into 3.2 for both kinetics and dynamics. This division of the 10-fold factors into default toxicokinetic and toxicodynamic factors offers the possibility of incorporating chemical-specific information and of exchanging the default sub-factors with data-derived factors [274] as has been done in the EC/SCF risk assessment of dioxins [10, 16]. However, this approach leaves the risk assessor with difficult tasks, such as evaluation of the quality and quantity of data required to change or lower the default factors [264].

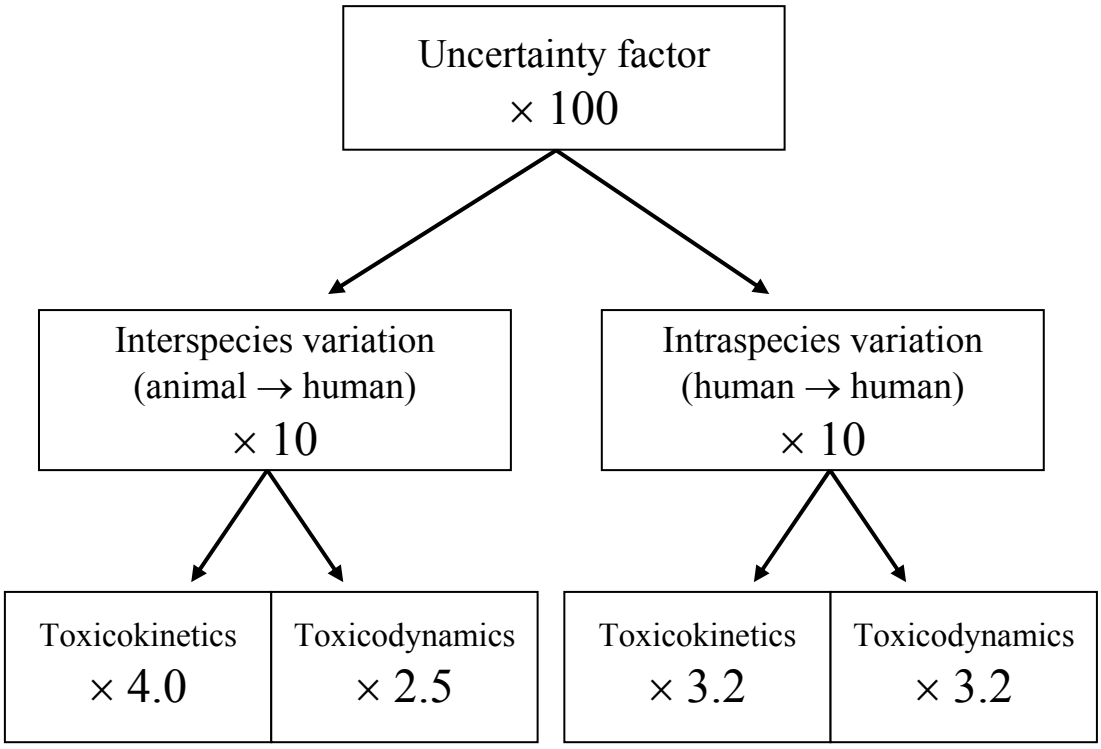

Figure 5. The commonly used uncertainty/assessment factor of 100 divided into sub-factors of different kinds of variability or uncertainty [272, 273].

Besides the commonly used assessment factor of 100, additional factors can be used depending on the quality of the data. Additional assessment factors can also be applied in cases when a NOAEL is lacking, as has been done in the EC/SCF risk assessment of dioxin [10, 16]. Such factors can also be justified in cases when data are lacking, e.g. from long-term studies, regarding effects in children, or when severe and irreversible effects have been observed, such as teratogenicity and non-genotoxic carcinogenicity. The use of assessment factors and the scientific basis for them has been described in a report from the Swedish Chemicals Inspectorate [275] [264].

Toxicokinetics – interspecies differences, animal vs man

In an effort to account for the large differences between elimination half-lives of TCDD in various animal species, the use of body burden instead of intake levels for interspecies dose extrapolation was introduced by WHO in the 1998 TDI evaluation [4]. The use of body burden as the dose metric is assumed to eliminate most of the interspecies kinetic variation, which is a significant part of the uncertainty in risk assessments of dioxins. However, this model uses some assumptions that will be critically discussed below.

Under steady state conditions, it is possible to describe the relation between intake and body burden as:

Intake × Absorption efficiency = Body burden × Elimination rate

Elimination rate is expressed as a first order elimination ($\ln(2)/t_{½}$) and absorption efficiency as the absorbed fraction of dose. Body burden is here defined as the concentration in the body, i.e. the amount of TCDD per kg body weight.

Both WHO and EU-SCF used a default model for TCDD with an absorption efficiency of 50 % and a half-life of 7.5 years in humans [4, 10, 16]. With this model it is possible to model an associated estimated human daily intake (EHDI) for every estimated body burden in experimental animals. However, the default values used for absorption and elimination may not be accurate when calculating the EHDI of TCDD or when extrapolating the EHDI from TCDD to other dioxins and dioxin-like PCBs.

As described in the chapter about toxicokinetics, the absorption value of 50 % is lower than most kinetic studies on dioxin-like compounds suggest. Most studies indicate absorption efficiency between 60 and 95 % (including studies in humans). The available data indicate no differences in absorption efficiency between species. We therefore suggest that an absorption efficiency of 80 % should be used when intake levels are calculated from body burdens.

Based on data on elimination rates reported by Flesch-Janys et al. [43], the half-life of 7.5 years used by WHO and EC/SCF seems to be a reasonably good estimate for an average TCDD elimination rate in humans at background exposure levels.

The model used by WHO and EU-SCF assumes that the same body burden in animals and humans also gives the same concentration in the target organ [4, 10, 16]. This is only the case if the human and animal body have a similar distribution of dioxin within the body. The distribution of dioxins in the body is mainly dependent on the amount of lipids within the tissues. Most of the dioxins are found in adipose tissue, and according to the International Commission on Radiological Protection (ICRP) the reference values for adipose tissue in adult humans are 25 and 37.5 %, for men and women, respectively [276]. The mice and rats used as laboratory animals normally have about 10 % of their body weight as adipose tissue [277-279].

TCDD levels in body lipids may be a better dose metric than the body burden for extrapolation between animals and humans, since levels in lipids give a more direct measure of the exposure of target organs for TCDD toxicity at low doses of exposure. TCDD levels in different lipid compartments of the body are assumed to be in equilibrium and the TCDD level in one lipid compartment therefore gives a measure of the levels in all other compartments, including blood. These are also likely to be in equilibrium with concentrations at the site of action, although it gives no information on the actual level. The partition coefficients seem to be stable at low doses. Only at higher doses, the hepatic levels of CYP1A2 influence the partition significantly by selectively binding TCDD and some other dioxin congeners.

Since humans tend to have a higher proportion of body fat than rodents, the use of a rodent body burden may in most cases underestimate the resulting estimated human daily intake (EHDI). In conclusion, we therefore suggest that TCDD levels in body fat should be used when extrapolation between rodents and humans is performed and that a human reference value of 20 % adipose tissue (at the lower end of the distribution for adults) should be used when the human body burden is calculated. In addition, we suggest the use of 80 % absorption and a half-life of 7.5 years. By doing this there is no need for an extra assessment factor compensating the toxicokinetic differences between animals and man.

Toxicokinetics – inter-individual differences, man vs man
The toxicokinetic determinants of dioxin and related compounds seem to depend on three major properties: lipophilicity, metabolism and binding to CYP1A2 in the liver. Important parameters that influence the inter-individual variation are therefore the amount of body fat, the metabolic activity, and the enzymatic binding capacity.

The human population varies in many biological processes that influence the kinetic behaviour of dioxin. The clearance half-life in the blood fat has been studied on an individual level among Yusho and Yucheng patients [46]**.** For pentaCDF, the average t½ was 1.1±0.1 years at concentrations >3 ppb, whereas at <3 ppb the t½ for pentaCDF was 7.5±4.0 years. A similar pattern was also observed for hexaCDF and heptaCDF. Within the Yusho and Yu-Cheng groups there was a gradual increase in half-life with age that may indicate a lower capacity of metabolism or increased storage capacity at older ages. In Seveso adults and Ranch hand veterans with high TCDD body burdens, TCDD showed a bi-phasic elimination [142]. The reason for the faster clearance at high body burdens may be related to enhanced metabolic activity and binding capacity of the CYP1A isoenzymes. At lower body burdens there was a considerable variation in t½ within the range of 2-16 years. Taken together, the results suggest that the individual variation in toxicokinetics (derived mainly from highly exposed men) can be predicted to be twofold between the average and the upper range of t½.

Seveso women eliminated TCDD slower (t½=9.8 years) than males (6.9 years) [142], a pattern that other authors attributed to increases in BMI among older women [280]. On average this gender difference results in EHDI values for women that are 30 % lower than for men, i.e. the intake needed to reach the same body burden decreases with increasing half-life.

Body composition (mass of body fat) varies between individuals. Females have a higher mass of body fat than males and for both sexes the amount increases with age. In males, body fat increases from about 20 % at the age of 20 to about 30 % at the age of 65. In females it increases from about 30 % to about 40 % [276]. There is also a large inter-individual variation in the population that is not sex or age dependent. Illnesses, pregnancies, physical activity, food habits etc. influence the kinetics of dioxins. Increases in the amount of body fat can cause a dilution of lipophilic contaminants and vice versa. However, increasing body fat is often related to a high intake of food that may or may not contain dioxin-like contaminants.

Metabolic activity may differ between persons. However, the exact mechanism of dioxin metabolism in humans is not well characterized, and the kinetic differences due to metabolism are difficult to interpret.

In summary, the variation of key parameters such as mass of body fat and elimination rate show significant individual differences. We therefore suggest that a safety factor should be used. The default factor of 3.2 for inter-individual differences in toxicokinetics seems to be reasonable. However, it should be noted that the relationship between dioxin intake and resulting dioxin levels in the body is complex and influenced by the level of exposure, amount of body fat, metabolism, and elimination half-life.

Toxicodynamics - animals vs humans

In earlier risk assessments on dioxins it was stated that most biological effects of dioxins appear to be mediated by the AhR [281, 282], which is indeed one of the corner stones of the TEF concept [2, 3]. At the same time, assessment documents point to extensive and important species differences in the functional response elicited by dioxins [283]. These differences could be due to a variety of factors including toxicokinetics, but also to toxicodynamic mechanisms such as receptor distribution and affinity, agonist action upon receptor binding etc.

In retrieving data on ligand-binding properties of the AhR and receptor interaction with dioxins, several experimental model systems have been used. In addition, data are also available on the ligand-binding specificity of the human AhR [282, 284, 285], and the results have been collected using a wide variety of tissues and primary/established cell-lines. The mean TCDD-binding affinity for the human AhR appears be lower than that observed in responsive mouse strains such as C67BL/6J [286]. However, a range of affinity values for human AhR exists that is similar in magnitude to the range of affinities observed between responsive and non-responsive mouse strain [286]. It could be shown that the range of binding affinities of TCDD for the human AhR generally is below, but overlaps, the AhR-binding affinities in rodent strains [287]**.**

Regarding CYP1A1 induction in human primary hepatocytes and HepG2 cells, the agonist potency of TCDD and other PCDD congeners was found to be lower by a factor of ten in the human cells than in rat hepatocytes and H4IIE cells [288, 289]. In Wistar rats and in mice with transplanted human thymus tissue (SCID mice), however, similar thymotoxic sensitivities to TCDD were reported [290]**.**

In recent publications, results are presented that tend to favour a less conservative view in the extrapolation of effect responses from animal to man. The human genome is reported to contain fewer than 40 % of the active dioxin-responding genes found in mice and rats [291]. Silkworth et al. [292] emphasised the differences between human and animal models in dioxin risk assessment, stating that CYP1A induction in human cells are about 10-1 000 times less sensitive to TCDD, PCB-126 and Aroclor 1254 than in rat and monkey cells. Lastly,

Moriguchi et al. [293] have used ”knock-in” DBA mice in which the native AhR in the genome was exchanged for the human AhR (hAhR). When TCDD was administered, a diminished induction of CYP genes was observed in the homozygous hAhR mice (DBA), as compared to the ”wildtype” DBA animals. These mechanistic studies indicate that humans are not more sensitive to TCDD than animals, but little is known about possible differences in toxicodynamic mechanisms after the stage of AhR binding and the relevance of CYP induction as a marker for toxicity.

A comparison of differences in toxicodynamics between humans and animals based on human epidemiological studies is difficult, since studies on variation in human sensitivity to dioxins include probable differences in both toxicokinetics and toxicodynamics. Epidemiological studies of cancer have not been able to draw firm conclusions on the causality of observed associations between dioxin exposure and effects, due to problems with exposure assessment and confounding. However, exposure assessment in the published epidemiological studies indicate that the occupationally and accidentally exposed human populations from Europe and the US are not much more sensitive than the animals used in the experimental cancer studies (see cancer section). In the most highly exposed cohort groups with increased cancer mortalities, estimated lipid levels of TCDD were within the same range as the levels causing increases in cancer incidence in animals. As discussed earlier in the cancer section, it is possible that the TCDD levels in occupationally exposed cohorts have been underestimated. This possibility further strengthens the suggestion that the individuals in the occupationally and accidentally exposed cohorts on average are not more sensitive than the most sensitive animals used in the experimental cancer studies.

It is even more difficult to draw conclusions about differences in toxicodynamics between animals and humans regarding immunological effects. The epidemiological studies of occupationally and accidentally exposed cohorts have not been able to draw conclusions about causality between exposure and observed effects on immune function. No epidemiological study has indicated severe immunosuppressive effects of high TCDD exposure, or clear-cut associations between dioxin exposure and immune-related disease. This indicates that humans are not more sensitive than animals regarding severe immunotoxic effects.

None of the epidemiological studies of highly exposed populations were, however, originally designed to comprehensively study immune function and more subtle immune system-related adverse effects on health. A few epidemiological studies on children and adolescents have shown significant associations between background body burdens of organochlorines, among them dioxins and dioxin-like substances, and changed immune status [218-220]. These studies indicate that children and adolescents may be sensitive to immunological effects of dioxins and dioxin-like substances, but firm conclusions cannot be

drawn due to co-exposure to non-dioxin-like substances and uncertainties about the causality of the dose-effect associations.

Regarding developmental effects, limited data on tooth development indicate that human sensitivity does not appear greater than that of the rat [226, 294].

To conclude, most mechanistic data on dioxin sensitivity and the cancer studies suggest that humans on average are not more sensitive than the most sensitive animals used in experimental studies. Thus, an assessment factor for inter-species variation in sensitivity (toxico-dynamics) is not needed. For other effects, e.g. immune and reproductive effects, however, knowledge on inter-species differences in sensitivity are lacking.

Toxicodynamics - human variability

Polymorphism in the Ah receptor gene is a possible mechanism behind differences in toxico-dynamics among humans. AhR polymorphism has a great influence on the sensitivity of rodents to adverse TCDD effects (see above, toxicodynamics animals-humans). Relatively few AhR-gene polymorphisms have been found in humans [295]. These occur mainly in the transactivating domain of the AhR, responsible for regulating expression of other genes [295]. Among humans a more than tenfold difference in the affinity of TCDD to the Ah receptor has been found, but this variation could not be associated to any known polymorphism in the human Ah receptor gene [295, 296]. Although the source of the large differences in Ah receptor affinity among humans is currently unknown, it could cause inter-individual variation in susceptibility to dioxins. Differences in induction of Ah receptor-dependent cytochrome P450 enzymes among humans have to some extent been connected to polymorphism in the Ah receptor, but results are not consistent [295].

Another possible mechanism for differences in dioxin sensitivity among humans is polymorphism in AhR-responsive genes, such as CYP1 genes [296]. A few studies indicate that certain genotypes of the polymorphic CYP1A1 gene are connected to an elevated risk of breast cancer after high PCB exposure [136-139]. Although the results are uncertain due to a small number of cancer cases, it has been speculated that CYP1A1 gene variants with greater inducibility by PCBs may lead to a greater carcinogen bioactivation and therefore a higher potential of cancer risk [139]. A study of a group of 62 Seveso subjects from the TCDD-exposed zones A and B and 59 subjects from the surrounding non-contaminated area found evidence of inter-individual variability of CYP1A1 and CYP1A2 induction by TCDD *in vitro* that could be explained by genetic variability in the CYP genes [297].

The magnitude of differences in susceptibility to TCDD and other dioxin-like compounds within the human population is not known. Mechanisms behind differences in human toxico-dynamics may be present at all stages from the binding of dioxins to the Ah receptor to the initiation of the adverse effects in tissues and organs. Studies of dioxin-induced chloracne in the Seveso population have suggested large inter-individual variation in sensitivity both within age groups and between age groups. Analysis of TCDD levels in a small number of serum samples collected shortly after the accident showed that individuals without chloracne had serum TCDD levels up to 10,400 pg/g lipid [298]. In chloracne patients the lowest detected TCDD concentration was 828 pg/g lipid, suggesting that the difference between the highest level not causing disease and the lowest levels causing disease was more than tenfold.

Chloracne was more frequent among younger individuals than among older [161] and chloracne was induced at lower serum TCDD levels among the younger patients [298]. Moreover, a strong association was found between plasma TCDD levels and chloracne among subjects with light hair colour [161]. This indicates that genetic factors may modify TCDD toxicity. It is not possible, however, to conclude that these differences were solely due to toxicodynamic variation, since inter-individual differences in exposure (dermal vs inhalation/oral) and toxicokinetics could be involved [161]. Other possible adverse human effects of TCDD and other dioxin-like compounds are not as easily detected on an individual basis as chloracne, and therefore little is known about possible differences in sensitivity within the human population of the world.

In summary, there are many uncertainties about inter-individual variability of toxicodynamics among humans. Polymorphisms in the Ah-receptor gene and in Ah-receptor responsive genes are possible sources of variability. Other possible sources of variability in sensitivity within the human population are sex differences and differences between children/adolescents and adults. A few studies indicate that variability may be more than tenfold. We suggest that the default factor of 3.2 should be used for possible inter-individual variation in sensitivity.

Calculations of TDI for non-developmental effects

In the present risk assessment the Benchmark dose (BMD) method was used in the calculation of a TDI for non-developmental effects of dioxin. Of the adverse (neoplastic) endpoints from the cancer studies (see Table 6) liver cholangio-carcinoma in the NTP rat study was used for the final calculation of TDI. Both hepatic tumours in the Kociba rat study and total malignant tumours of all organs in the NTP study resulted in slightly lower BMDL levels. However, the BMDL

for liver cholangiocarcinoma was considered a more reliable starting point for the TDI calculation. In addition, the NTP study was considered of higher quality than the Kociba study, for example because of the use of more animals per group and more dose levels. The BMDL05 level for liver cholangiocarcinoma in the NTP study was estimated to be 1.50 ng TCDD/g fat at steady state. From the BMDL05 concentration of 1.50 ng/g fat the corresponding human body burden was estimated to 300 ng/kg bw, assuming a 20 % body fat in the human. By using a one compartment kinetic model and the first order kinetics with 80 % absorption and a half-life of 7.5 years as constants, the estimated human daily intake (EHDI) was calculated to 95 pg/kg bw/day. Assessment factors were then applied to this value to calculate the TDI (Table 7).

We have calculated the TDI for three different scenarios using different sets of assessment factors. Similarly to the NOAEL approach we used the BMDL05 as an estimate of the threshold of no adverse effects as a base for the calculation of the non-developmental TDI and thus no extra factor was needed for extrapolation from LOAEL. The EU/SCF adjusted for interspecies differences in toxicokinetics by using the body burden approach, i.e. expressed the body burden of TCDD at the LOAELs/NOAELs for developmental effects as ng TCDD/kg body weight. We used the TCDD level in body fat for this extrapolation. The three different scenarios for the calculation of a TDI from this BMDL are shown in Table 7. The corresponding EHDI has been calculated as the long-term intake that will result in the particular body burden at steady state assuming an absorption factor of 80 % and a half-life of 7.5 years.

In the first scenario (scenario 1), the default assessment factor of 3.2 was applied in order to account for possible individual differences in toxicokinetics among humans. Assessment factors for differences in toxicodynamics between animals and humans and within the human population were not used based on the assumption put forward by SCF and JECFA that the most sensitive humans are not more sensitive to TCDD effects than rats. The total assessment factor of 3.2 in scenario 1, to account for uncertainties about of toxikokinetic differences in humans, is similar to the assessment factor used by the SCF and JECFA in their risk assessment of developmental effects of TCDD [4, 10, 16, 98]. The resulting TDI in scenario 1 after the use of the toxicokinetic assessment factor was 29.7 pg/kg/d (Table 7).

In scenario 2, we used the default assessment factor for toxicodynamics differences in humans (3.2) in addition to the assessment factor for differences in kinetics among humans. This scenario is based on data suggesting a large difference in human sensitivity to dioxins. This variation could both be due to toxicokinetic and toxicodynamic differences among humans, but it is not possible to separate the contribution of the two factors. As a result, assessment factors for both kinetic and dynamic differences among humans were applied to the intake at

BMDL05. The resulting TDI in scenario 2, after the use of the assessment factor of 10, was 9.5 pg/kg/d (Table 7).

In scenario 3, an additional assessment factor of 5 was used, accounting for the seriousness of the cancer effect. This additional safety margin may also be motivated since the BMDL05 represents an exposure level that in the worst case could result in a 5 % increase in cancer risk. Moreover, in the critical cancer study on rats, non-neoplastic effects (that may or may not lead to cancer or other severe effects) were observed at dose levels below the BMDL05. The resulting TDI in scenario three after the use of the total assessment factor of 50 was 1.9 pg/kg/d (Table 7).

Table 7. Calculation of Tolerable Daily Intake (TDI) and the corresponding concentration in body fat for non-developmental effects of TCDD following three different scenarios. All scenarios are based on the Benchmark dose level (BMDL05) of 1.5 ng/kg fat for liver cholangiocarcinoma modelled from the cancer study performed by the National Toxicology Programme [108]. Corresponding estimated human daily intake (EHDI) was calculated to 95 pg/kg bw/day and assessment factors was applied to this value to calculate the TDI[a].

Scenario	Description	EHDI (pg/kg/d)	AF	TDI (pg/kg bw)	Corresponding conc (pg/g fat)
1[b]	Includes AF for human differences in toxicokinetics	95	3.2	29.7	500
2	Includes also AF for human differences in toxicodynamics	95	10 (3.2*3.2)	9.5	150
3	Includes also AF for severity of effect	95	50 (3.2*3.2*5)	1.9	30

[a] EHDI is calculated using a one compartmental kinetic model with the following assumptions: Amount of body fat in rats = 10 %, amount of body fat in humans = 20 %, absorption rate in humans = 80 %, half-life in humans = 7.5 years
[b] Similar to the AF used by EC/SCF [16]

Application of TEQ

Our risk assessment, and those of WHO and EU, are based on studies of TCDD toxicity. In the WHO-TEF system TCDD is used as the reference substance and accordingly always has a TEF of 1. Thus, the TDIs are not influenced by revisions of the WHO-TEFs, which occurred recently [3].

Similar to the WHO [4] and EC/SCF [10, 16] risk assessments we considered it appropriate to extend the TDI for TCDD to all 2,3,7,8-substituted PCDDs and PCDFs and dioxin-like PCBs, expressed as WHO-TEQ [2, 3].

A number of uncertainties exist in the use of the TEF concept for human risk assessment [3, 4, 10]. However, pragmatically it remains the most feasible approach. Use of TCDD alone as the only measure of exposure to PCDDs, PCDFs and dioxin-like PCBs would severely underestimate the risk to humans from exposure to these compounds. However, the TEF tool needs further refinement.

In the risk assessment of dioxins by EC/SCF [10] it was considered essential that the differences in half-lives between TCDD and the other dioxins and dioxin-like PCBs are either small or accounted for in the establishment of the WHO TEF values. The assumption that kinetic differences between congeners are accounted for within the WHO-TEFs depends on both the amount of data and the quality of data that the TEFs are based upon. TEFs should preferably be based on long-term *in vivo* studies. However, such studies are not always available and thus the TEF does not fully account for differences in half-lives between congeners. In addition, it is also assumed that the relationship between half-lives of different congeners is the same for rats and humans. Evidence for such a similar pattern in half-lives has not been reported.

Both WHO and EU-SCF use a default model for TCDD with a half-life of 7.5 years [4, 10]. Beside TCDD the four most abundant (expressed as TEQ contribution) PCDD/Fs in human milk and Baltic fish are 2,3,4,7,8-PCDF, 1,2,3,7,8-PCDD, 1,2,3,6,7,8-PCDD, and PCB126 [48, 299-301]. These four congeners contribute to about 80 % of the total TEQ content. The median half-lives in humans for these four congeners were estimated to be considerably longer than for TCDD. This clearly shows the need for congener specific kinetic data.

Discussion

The aim of our risk assessment was to identify the critical health effects for humans exposed to dioxins during childhood and adulthood, and to find a tolerable exposure level for these stages in life. Animal studies show that adults are less sensitive to dioxin exposure than the developing fetus. In the cancer studies on rats, lipid levels of TCDD at NOAEL were estimated to 540-2000 pg/g lipid (see Table 4). In the WHO/JECFA risk assessment of 2002 [98] a NOAEL maternal body burden of 13 ng TCDD/kg body weight was estimated for adverse effects on reproductive development in male rats after *in utero* exposure [7]. Assuming a lipid content of 10 % in the rat a NOAEL TCDD lipid concentration of 130 pg/g lipid is reached. Similarly, maternal LOAEL TCDD lipid concentrations for developmental effects and immunosuppression in offspring ranged from 250-500 pg TCDD/g lipid [98]. In humans, studies of children born by mothers poisoned by PCBs and PCDFs in the Yu-Cheng incident have clearly suggested that the developing fetus is the most sensitive life stage also in humans [302].

In the present risk assessment of non-developmental effects of dioxin, the studies used as the basis for calculation of TDI were all performed with adult exposure. There is a lack of animal and human data to allow for an assessment of risks during exposure at young ages. Kinetic data may point towards a lower sensitivity in children and adolescents. Because of the long half-lives of dioxins, steady-state is achieved very slowly, and in children dioxins might be diluted because of body growth and a possible shorter half-life. It was, however, indicated from studies of chloracne and sex ratios of offspring from Seveso that children and adolescents in some cases may be more sensitive to dioxin exposure than adult [161, 251].

Our TDI calculations were based on an oral absorption factor of 80 % when the estimated human daily intake (EHDI) was calculated, instead of the 50 % absorption used by WHO and EU/SCF. The higher absorption factor is in agreement with available animal and human data (see Toxicokinetic section). In the WHO and EU/SCF risk assessments, the body burden of TCDD, expressed per kilo body weight, was used in interspecies extrapolation pf toxico-kinetics. We did the toxicokinetic interspecies extrapolation by using TCDD concentrations in fat. After initial distribution, the adipose tissue and liver is reported as the predominant storage sites of dioxins. At high concentrations there is a significant binding to CYP1A2 proteins in the liver. However, at low constant doses there is a steady state with constant partition coefficients between the body compartments and most of the given dose is found in the lipid compartments of various organs in

the body. Dioxin levels in body lipids may therefore be a better dose metric for extrapolation between animals and humans than body burden. Since humans tend to have a higher proportion of body fat than laboratory rats and mice, we used the assumption of 20 % body fat for humans and 10 % for laboratory rodents as constants when estimating a corresponding human daily intake.

Cancer was considered the most critical and sensitive adverse effect of TCDD in animal studies of non-developmental exposure. TCDD is not genotoxic and therefore it can be expected that no cancer risk occurs when the exposure level is below the practical threshold of the dose-response curve [303, 304]. The benchmark modelling of the animal cancer data resulted in a BMDL05 of 1500 pg TCDD/g fat (corresponding to 300 ng/kg body weight in humans), and a EHDI of 95 pg/kg bw. At this exposure level the cancer risk in rats was increased by 5 % above background.

We have presented three different scenarios for the use of assessment factors in the calculation of a non-developmental TDI. In all scenarios, no assessment factor was used for toxicokinetic extrapolation between rats and humans. This extrapolation was taken care of by the use of body burdens (here levels of TCDD in body lipids). In accordance with WHO and EU expert groups [4, 16] no assessment factor for toxicodynamic extrapolation between rats and humans was applied. In the present risk assessment this was based on the cancer studies on humans that suggest that humans on average are not more sensitive than rats to cancer effects. In the most highly exposed cohort groups with slightly increased cancer mortalities, estimated lipid levels of TCDD were within the same range as the lowest levels in animals causing measurable increases in cancer incidence. As discussed earlier in the cancer section, it is possible that the TCDD levels in occupationally exposed cohorts have been underestimated. This possibility further strengthens the suggestion that the individuals in the occupationally and accidentally exposed cohorts on average are not more sensitive than the most sensitive animals used in the experimental cancer studies.

In **scenario 1**, no assessment factor for toxicodynamic differences among humans was applied. This approach was used by the WHO and EU expert groups, since they concluded that the most sensitive humans are not more sensitive than the most sensitive animals [4, 16]). Thus in scenario 1 the only assessment factor used was a factor for toxicokinetic differences in humans (3.2x) resulting in a TDI of 29.7 and a corresponding body burden of about 500 pg/g lipid.

We do, however, believe that an assessment factor for differences in toxicodynamics among humans should be applied on the BMDL05. Both mechanistic and epidemiological studies suggest that the variation in sensitivity among humans is substantial (see Toxicodynamics-interindividual differences). In some

cases this difference in sensitivity probably includes both differences in toxicodynamics and toxicokinetics. Therefore, in **scenarios 2 and 3** an additional assessment factor for differences in toxicodynamics within the human population (factor 3.2) was applied. In **scenario 3** an assessment factor of 5 was added to account for the severity of effect (cancer) and the fact that the BMDL05 represented an exposure level causing a 5 % increase in cancer incidence. **Scenarios 2 and 3** resulted in a TDI range of 2-10 pg TCDD/kg bw, corresponding to a human body burden of 30-150 pg TCDD/g lipid (assuming 20 % body fat).

As discussed above, the assessment factor accounting for differences in toxicodynamics among humans was not used by the EU/SCF in their calculation of a developmental TDI. A use of this additional assessment factor in the risk assessments of *in utero* exposure performed by the EU/SCF would result in a lower developmental TDI than the current EU/SCF TDI. However, the application of assessment factors may differ depending on the critical effects used and the quality of data.

A key question for the risk managers is: does the current background dioxin exposure pose a cancer risk among humans? A few efforts have been made to determine if there are detectable cancer risks among humans at background exposure levels in the exposure range in scenarios 2 and 3, i.e. 2-10 pg TEQ/kg bw/day. A modelling of ED01 for cancer risks (dose associated with a 1 % excess in lifetime risk) [305] was performed on data from the US chemical workers cohort [306], and the two German occupational cohorts [307, 308]. ED01 was estimated to range from 1.8-7.1 pg TCDD/kg bw/d, which is within our TDI-range for cancer from scenarios 2 and 3. The outcome of such modelling of cancer risks is, however, dependent on the assumptions made from the data used and on the different models used. It was noted by Portier [305] that the assumptions about timing of TCDD exposure during employment, dose metrics, and dose-response relationships used in the analysis could result in considerable overestimation of risks.

In a meta-analysis, which also included the US chemical workers cohort and the two German occupational cohorts [118], a linear dose-response extrapolation from high dose human cancer risks to low dose risks indicated a 1 % increase in lifetime risk of cancer mortality at a TCDD intake of 45 pg TCDD/kg/d. This ED01 is 6-25-fold higher than the ED01s modelled by Portier [305], illustrating the uncertainties behind modelling of cancer risk among humans based on epidemiology. In the same modelling effort, no increase in total cancer risk compared to background could be detected at an exposure level of 6 pg/kg/day [118]. This lack of increase in cancer risk could be due to either a true lack of effect at low exposure levels or to inherent limitations of the methods used.

To our knowledge, no epidemiological study has shown consistent and clear increases in cancer risks at current background exposures to dioxins and dioxin-like PCBs in humans. Among fishermen and their wives with potentially high exposures to dioxins from contaminated fish from the Baltic Sea (men: N=2896; women: N=1989), no increase in overall cancer mortality and incidence was detected in comparison with the expected mortality among the general population [129, 309]. In this population of fishermen average lipid levels of dioxins of approximately 90 pg TEQ/g lipid have been reported, including PCDD/DFs and non-*ortho* PCBs [310]. This average TEQ level corresponds to an average intake of about 6 pg TEQ/kg bw/day. The majority of the total-TEQ intake most probably originates from Baltic Sea fish. Mono-*ortho* TEQ levels contribute approximately 1/8 to total TEQ levels in this type of fish [311]. The additional contribution of mono-*ortho* dl-PCBs to TEQ intake among the Swedish fishermen results in an average intake in the upper range of the TDIs in scenarios 2 and 3 (2-10 pg/kg bw/day).

In a case-control study of soft tissue sarcoma (STS) with study participants from the general population in Finland, no increased STS risk with increased body burden of PCDD/DF/PCB TEQ could be detected [124]. In this population the total-TEQ level ranged from 5 to 200 pg total-TEQ/g lipid, corresponding to intakes of approximately 0.3 to 13 pg TEQ/kg bw/day. Moreover in the numerous breast cancer studies, where levels of PCB in body lipids could be regarded as a surrogate for TEQ levels (see cancer section), the background PCB exposure has not been associated with an increased cancer risk.

In epidemiological studies it is not possible to conclude that there is no health risk at all associated with a certain exposure, since very low risks are difficult to detect. The cancer epidemiological studies nevertheless suggest that the cancer risks are at least very low at background dioxin exposure levels within the range of the TDIs from scenarios 2 and 3 (2-10 pg/kg bw/day). At exposure levels in the lower range of the TDI-interval, cancer risks could most probably be regarded as non-existing.

The current median PCDD/DF/PCB TEQ intake among female and male adults in Sweden (17-75 years old) has been estimated to 1.1 pg TEQ/kg/d, based on consumption data from the national food consumption survey Riksmaten 1997-98 [20]. The intake level at the 95 percentile (2.9 pg/kg/d) was estimated to be slightly higher than the most conservative TDI estimated in scenario 3. This shows that the majority of the adult population in Sweden have intake levels that should be considered to be safe regarding potential cancer risks, based on current know-ledge about dioxin toxicity. The consumption of fatty fish from the dioxin-contaminated areas in Sweden was low, with a median consumption of 1 serving per month and a consumption at the 95 percentile of 3 servings per month [312].

Based on data from a national food consumption survey performed in 2002, median intakes of TEQ among Swedish children in ages 4-12 years has been estimated to 1.2-2.3 pg TEQ/kg/day, with higher intakes per kg body weight for younger children [313]. This is mainly due to a higher food consumption rate per kilo body weight among younger children. The 95 percentile intake was estimated to 3-5 pg TEQ/kg/d. Intake levels are thus below or within the lower TDI range for scenarios 2 and 3. The relationship between TEQ intake and the resulting TEQ levels in body lipids are not well known in growing children. Modelling efforts have been made indicating that the rapid increase in body weight and height results in fairly constant, or even decreasing lipid levels of TEQs in children of increasing age [280]. This suggests that the slightly higher intake levels among children than among adults is compensated for by the rapid body growth in children. Increased cancer risks are dependent on life-long exposures to elevated dioxin levels. Consequently, the intake levels of TEQ among Swedish children most probably pose no cancer risk, although intake levels for a short period in life are slightly higher than the intakes among adults. The majority of the children (70 %) did not eat the fish included in the current fish consumption advisory regarding fish with high dioxin concentrations in Sweden.

In a study of temporal trends between 1996 and 2004 of dioxins in breast milk among Swedish women of ages 21-41 years (N=153), median total TEQ level in breast milk lipids was 16 pg TEQ/g lipid and the maximum level measured was 39 pg TEQ/g lipid [314]. Dioxins in breast milk lipids are in equilibrium with dioxins in body lipids. A comparison can therefore be made between dioxin levels among the Swedish women and average TCDD levels in occupationally exposed sub-groups with the highest TCDD exposures (approx. 1500 pg TCDD/g lipid), for which slight increases in cancer risks also were suggested (Table 4). There is a large margin between the median body burden among the young Swedish women and the body burdens in the occupationally exposed groups (94-fold). Even among the women with the highest measured background exposure, margins to the occupational body burdens were considerable (38-fold). The body burdens of dioxins among young Swedish women decreased with 6.6 % per year between 1996 and 2004 [314]. There was no indication that this decrease in body burdens among young women was levelling off at the end of the study period. This shows that the background dioxin body burdens in Sweden most probably will continue to decrease in the near future.

The only recent risk assessment on non-developmental exposure to dioxins, aside from the present one, has been published by the UK Food Standard Agency [18]. They based their assessment on a body burden LOAEL from the Kociba cancer study in female rats. Their calculation of a ”Guideline Level for Long-Term Exposure” was in accordance with the assessment factors and kinetic parameters used by EU-SCF and resulted in a Guideline Level of 8 pg TEQ/kg body weight and day. This is within the upper range of the TDIs in our scenarios 2 and 3.

However, our risk assessment differs from the UK FSA (as well as the EU-SCF) risk assessment in several ways, for instance regarding the use of critical study, BMD-approach, interspecies extrapolation on lipid basis, absorption factors and assessment factors.

In conclusion, in the risk assessment of non-developmental effects of dioxins we identified cancer as the most sensitive critical effect based on results from animal studies. Cancer effects in animals, caused by non-developmental dioxin exposure, occur at higher exposure levels than effects after developmental exposure. Our risk assessment differed in several ways from the recent risk assessments of EU/SCF and WHO. We used body lipid levels of TCDD in the toxicokinetic extrapolation between animals and humans instead of the body burden, expressed as amount of TCDD per kilo body weight. Moreover, a higher oral absorption efficiency of TCDD was used by us (80 % vs 50 %) when the estimated human daily intake (EHDI) was calculated. In our risk assessment, we also used an additional assessment factor accounting for uncertainties about possible differences in sensitivity (toxicodynamics) among humans (scenario 2). In the TDI calculation we also illustrated the impact of applying an extra assessment factor for the severity of the critical effect (scenario 3). The resulting TDI range in scenarios 2 and 3 was estimated to 2-10 pg TEQ/kg body weight/day. Children and adults in the general Swedish population have exposure levels that are lower or only slightly higher than the most conservative TDI (2 pg/kg body weight/day). Based on current knowledge on dioxin toxicity, this suggests that the majority of the Swedish population have long-term intake levels that could be considered as safe regarding non-developmental dioxin exposure. Concentrations of dioxins in certain types of fatty fish from the Baltic Sea are however still high but the consumption of these types of fish is low among the general Swedish population. Nevertheless there may still be a need for consumption advisories regarding fish with high dioxin concentrations. Such advisories would inform consumers, who have a very high consumption of dioxin-laden fish, on possible measures to decrease their dioxin exposure. However, a health-based risk-benefit analysis of high consumption of dioxin-laden fish is needed before final conclusions can be made about how consumption advisories should be formulated. The most effective long-term and sustainable measure in order to ensure tolerable dioxin levels in food is to control dioxin emissions from important primary and secondary sources.

Data gaps and research needs

Several data gaps can be identified regarding the science on which our risk assessment is based. The research needs are mainly related to five research areas; effect studies, epidemiology, dose-response modelling, kinetic models and the TEF concept.

Effect studies (experimental and epidemiological studies)

There is a special need for knowledge about critical effects, for which current data were not sufficient to be used for TDI calculations.

- Immunotoxicity. Better dose-response data in animals, epidemiological studies regarding endpoints such as allergy, vaccination response etc.
- Endometriosis. Further epidemiological studies needed as well as mechanistic studies to confirm causality.
- Bone and teeth effects. Further studies are needed to explore dose-response relationships.
- Endocrine and reproductive effects. Research on effects in animals exposed early after birth. Epidemiological studies of exposed children and adolescents.
- Fertility. Epidemiological studies are required although difficult to design.
- Cardiovascular disease. Animal studies are needed to elucidate if the effects on the heart and circulatory system, seen in a few experimental studies, may cause adverse health effects.
- Diabetes. Prospective epidemiological studies are needed in order to confirm the associations observed in retrospective studies.

For all the effects mentioned above, studies of low-dose effects and dose-response relationships are critical. For all sensitive effects, including cancer, mechanistic studies are required in order to reduce uncertainty in extrapolation of risk from animals to the most sensitive human individuals.

Epidemiological studies

As mentioned above, there is a lack of knowledge about possible human effects after exposure during childhood and adolescence. Further epidemiological studies of these age groups in highly exposed cohorts (Seveso, Yusho, Yu-Cheng etc.) are important. Moreover, comparative studies of sensitivity in prenatal, adolescent and adult stages are needed. There is information on large inter-individual variation in sensitivity for chloracne, but more data on the variability for other effects is critical for the risk assessment.

Dose-response assessment

Further refinement of the BMD methodology is needed. Important aspects include curve fitting in the low-dose area, and dose-response modelling of continuous endpoints, e.g. body and organ weights, and biochemical parameters.

Kinetic models

The kinetic aspects are very important in dioxin risk assessment. This report shows several points related to kinetics in the current EC/SCF risk assessment that needs improvement Research that would help to improve the risk assessment includes kinetic modelling of groups of the population (probabilistic modelling), the influence of body fat content on kinetics and extrapolation between animals and humans, and half-lives of congeners other than TCDD in the low-dose range.

TEF concept

In addition to the above four areas, we conclude that the TEF scheme is an important component in the risk assessment and risk management of dioxins. Thus, the TEF concept as well as the individual TEF values, which have several limitations, should be refined as much as possible and re-evaluated regularly. Important aspects include an appropriate data base of useful studies and refined calculations of REP and TEF, preferably using BMD methodology.

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Rapporter som utgivits 2006

1. Mikroprofil Gris – Kartläggning av mikroorganismer på slaktkroppar av M Lindblad
2. Nyckelhålet för spannmålsprodukter av A Laser Reuterswärd
3. Interkalibrering av laboratorier. Mikrobiologi – Livsmedel, januari 2006 av C Normark och K Mykkänen
4. Studie av förstföderskor – Organiska miljögifter hos gravida och ammande. Del 1 Serumnivåer av A Glynn, M Aune, P O Darnerud, S Atuma, S Cnattingius, R Bjerselius, W Becker och Y Lind.
5. Kontroll av restsubstanser i levande djur och animaliska livsmedel – Resultat 2005 av I Nordlander, H Green och I Nilsson
6. Proficiency Testing – Food Chemistry, Nutritional Components of Food, Round N-37, by L Merino and M Åström
7. Proficiency Testing – Food Chemistry, Trace Elements in Food, Round T–12 by C Åstrand and L Jorhem
8. Krav på livsmedelsföretagarna – Utbildning i livsmedelshygien
9. Interkalibrering av laboratorier. Mikrobiologi – Livsmedel, april 2006 av C Normark och K Mykkänen
10. Interkalibrering av laboratorier. Mikrobiologi – Dricksvatten 2006:1, mars av T Šlapokas och C Gunnarsson
11. Rapportering om livsmedelstillsyn 2005 – Tillsynsmyndigheternas rapportering om livsmedelstillsyn av D Rosling
12. Rapportering av dricksvattentillsyn 2005 – Tillsynsmyndigheternas rapportering om dricksvattentillsyn av D Rosling
13. The Swedish Monitoring of Pesticide Residues in Food of Plant Origin: 2005, EC and National Report by A Andersson, A Jansson and A Hellström
14. Kontroll av svenska musselodlingar av I Nordlander
15. Studie av förstföderskor – Organiska miljögifter hos gravida och ammande. Del 2 Bröstmjölksnivåer samt korrelationer mellan serum- och bröstmjölksnivåer av S Lignell, A Glynn, M Aune, P O Darnerud, R Bjerselius och W Becker
16. Proficiency Testing – Food Chemistry, Nutritional Components of Food, Round N-38 by L Merino and M Åström
17. Proficiency Testing – Food Chemistry, Vitamins in Foods, Round V-4 by H S Strandler and A Staffas
18. Förslag till framtidens nyckelhålsmärkning i storhushåll – certifieringssystem och nya kriterier av U Bohman och A L Reuterswärd
19. Riksprojekt 2005: Centralt producerad mat till särskilt och enskilt boende - mikrobiologi och tillämpning av M Lindblad och A Westöö
20. Svenska barns matvanor 2003 – resultat av enkätfrågor av W Becker och H Enghardt Barbieri.
21. Interkalibrering av laboratorier. Mikrobiologi – Dricksvatten 2006:2, september av T Šlapokas, C Gunnarsson och M Foucard
22. Proficiency Testing – Food Chemistry, Trace Elements in Food, Round T–13 by C Åstrand and L Jorhem
23. Interkalibrering av laboratorier. Mikrobiologi – Livsmedel, oktober 2006 av C Normark, K Mykkänen, I Tillander och C Gunnarsson

Rapporter som utgivits 2007

1. Algtoxiner i avsaltat dricksvatten.
2. Nationellt tillsynsprojekt 2006 om livsmedelsmärkning.
3. Indikatorer för bra matvanor av W Becker.
4. Interkalibrering av laboratorier. Mikrobiologi – Livsmedel, januari 2007 av C Normark och K Mykkänen.
5. Proficiency Testing – Food Chemistry, Nutritional Components of Food, Round N-39 by L Merino and M Åström.
6. Nutrient Analysis of Dairy Foods and Vegetarian Dishes by M Arnemo, M Arnemo, S Johansson, L Jorhem, I Mattisson, S Wretling and C Åstrand.
7. Proficiency Testing – Food Chemistry, Trace Elements in Food, Round T–14 by C Åstrand and L Jorhem.
8. Riskprofil – Yersinia enterocolitica av S Thisted Lambertz.
9. Riskvärdering av persistenta klorerade och bromerade miljöföroreningar i livsmedel av E Ankarberg, M A, G Concha, P O Darnerud, A Glynn, S Lignell och A Törnkvist.
10. Riskvärdering av metylkvicksilver i fisk av K Petersson-Grawé, G Concha och E Ankarberg.
11. Risk assessment of non-developmental health effects of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and dioxin-like polychlorinated biphenyls in food by A Hanberg, M Öberg, S Sand, P O Darnerud and A Glynn.

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