Compilation of EU Dioxin Exposure and Health Data

Task 8 – Human toxicology

Report produced for

European Commission Environment

UK Department of the Environment, Transport and the Regions (DETR)

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Executive Summary

Dioxins are widespread environmental pollutants all over the world and are highly toxic. At present, exposure to dioxins in the general population of the European Union (EU) is at a level where subtle health effects might occur and it is, therefore, of utmost importance that the assessment of health risk is improved. Over recent years a vast number of research reports has been published on the toxicity of dioxins and in particular the most toxic dioxin, TCDD. This report reviews the toxicological effects of dioxins, recent assessments of health risk and exercises to set Tolerable Daily Intakes (TDIs) for dioxin-like compounds.

Dioxin-like compounds elicit a broad spectrum of responses in **experimental animals**. Among these effects are:

- liver damage (hepatoxicity);
- suppression of the immune system (immunotoxicity);
- formation and development of cancers (carcinogenesis);
- abnormalities in foetal development (teratogenicity);
- developmental and reproductive toxicity;
- skin defects (dermal toxicity);
- diverse effects on hormones and growth factors;
- and induction of metabolising enzyme activities (which increases the risk of metabolising precursor chemicals to produce others which are more biologically active).

Cancer was for long considered as the critical effect, i.e. the most sensitive effect, of dioxin exposure. However, in recent years, the foetus and newborn offspring of several species have been shown to be particularly sensitive to TCDD, resulting in effects on reproduction, immune function and behaviour.

In humans effects associated with exposure to dioxins are mainly observed in accidental and occupational exposure situations. A number of cancer locations, as well as total cancer, have been associated with exposure to dioxins (mostly TCDD). In addition, an increased prevalence of diabetes and increased mortality due to diabetes and cardiovascular diseases have been reported. In children exposed to dioxins and/or PCBs in the womb, effects on neurodevelopment and neurobehaviour (object learning) and effects on thyroid hormone status have been observed at exposures at or near background levels. At higher exposures, children exposed transplacentally to PCBs and PCDFs show skin defects, developmental delays, low birth-weight, behaviour disorders, decrease in penile length at puberty, reduced height among girls at puberty and hearing loss. It is not totally clear to what extent dioxin-like compounds are responsible for these effects, when considering the complex chemical mixtures to which human individuals are exposed. However, it has been recognised that subtle effects might already be occurring in the general population in developed countries, at current background levels of exposure to dioxins and dioxin-like compounds and, due to the high persistence of the dioxin-like compounds, the concentrations in the environment, as well as in food, will only decrease slowly.

In 1998, the World Health Organisation European Centre for Environment and Health (WHO-ECEH) and the International Programme on Chemical Safety (IPCS) gathered a group of international experts in order to perform a health risk assessment of dioxin-like compounds. The resulting risk assessment was based on the most up-to-date knowledge and information regarding critical effects (including developmental, reproductive, hormonal, immune system and neurobehavioural effects), dose-response relationships and quantitative risk extrapolation. A Tolerable Daily Intake (TDI) of 1-4 pg WHO-TEQ/kg body weight (including PCBs) was recommended. In common with all recent risk assessments, WHO and IPCS support the use of the TEF-scheme in the risk assessment of PCDDs, PCDFs, as well as dioxin-like PCBs.

The following priority actions are recommended in order to reduce the health risk from exposure to dioxin-like compounds across the EU:

Member States should be encouraged to:

- apply the WHO recommended TDI of 1-4 pg WHO-TEQ/kg/day;
- include both dioxins and dioxin-like PCBs in the TDI for dioxin-like compounds;
- reduce as far as possible the discharge of dioxins to the environment;
- identify highly exposed groups most likely to be at risk of damage from dioxin contamination;
- investigate the need for establishing dietary recommendations for certain foodstuffs.

Effort should be committed to reducing the uncertainty associated with the health risk assessment by carefully targeted research into:

- dose-response relationships, including no adverse effect levels for the developmental effects in animals;
- a more reliable and complete mechanistic understanding and support for the applicability of the TEF concept to the critical effects, i.e. developmental effects of PCDD, PCDF and PCB exposure;
- epidemiological follow-up on reproductive, neurobehavioural, immune system effects, as well as cancer in children exposed to dioxin-like compounds in the womb. These studies should include exposure analysis in order to describe the dose-response relationships of the effects.

Contents

1. Introduction

Dioxins are widespread environmental pollutants all over the world and, since they are highly toxic compounds, it is of utmost importance to improve the assessment of health risk. At present, exposure to dioxins in the general population of the European Union (EU) is at a level where subtle health effects might occur.

This report describes work undertaken to review the toxicological effects of dioxins and to identify work of relevance to Europe on the impacts of dioxin exposure to humans. It also seeks to establish whether there is sufficient information available to set safe tolerable daily intakes (TDIs). The following sections give an overview of the possible effects of dioxins in humans, based on experimental studies in animals as well as epidemiological studies in humans. In addition, the most recent health risk assessments for dioxins and the tolerable daily intakes are reviewed and discussed. Additional technical detail, a glossary of terms and abbreviations and a reference list are provided in the Technical Annex.

Toxicology can be defined as the study of the harmful effects of chemicals upon biological systems. This report deals with human toxicology. However, besides epidemiological studies in humans, the knowledge on human toxicology is mostly based upon extrapolation from studies in experimental animals (i.e. mammals).

In the context of this study, the relevance of toxicological research is that it offers the prospect of carrying out risk assessments for human individuals exposed to particular levels of contamination, based on the animal-derived mechanisms. These studies may, in turn, be used as a basis for recommending TDIs that will protect humans from the toxic effects of dioxins. From the health risk point of view it is relevant to include**,** besides dioxins, other dioxin-like environmental contaminants in the risk assessment. This group of chemicals includes, for example, the dioxin-like polychlorinated biphenyls (PCBs). However, this report is primarily focussed upon the toxicity of the polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs).

The information presented in the following sections has been obtained from an extensive review of the literature and through discussions with individuals, in Europe and North America, actively involved in relevant research or policy development.

2. Overview of Toxicological and Epidemiological Studies

Over recent years a vast number of research reports has been published on the toxicity of dioxins and in particular the most toxic dioxin, TCDD. The following sections review the current understanding of the ways in which dioxins act to damage organisms at a cellular level and at the level of the individual. The review is based upon experimental animal (i.e. mammalian) toxicology studies, and epidemiological human studies. Epidemiology measures the occurrence of particular diseases in broad human populations and correlates them with environmental exposure to various potential causative agents.

Dioxins cause a broad spectrum of different effects in several organs and tissues. This review, however, is mainly focussed on the critical effects of dioxin exposure, i.e. the effects occurring at the lowest exposure. These are the effects most relevant for human risk assessment and those which might occur in the general population after background dietary exposure to dioxins. A further section reviews the issues of assigning toxic equivalency factors (TEFs) to different dioxin congeners for the purposes of health risk assessment. These sections, inevitably, have much in common with the equivalent parts of the report on Task 7 - Ecotoxicology and some information has been included in both reports for completeness.

2.1 MODE OF ACTION

The basic influence of a damaging substance on an organism takes place at the molecular level. This influence, if strong enough, subsequently impacts upon the cellular, tissue and organ levels within the individual organism. Work on the molecular and cellular effects of dioxins to date suggests that the way in which they act is broadly the same. This is important, because it allows assumptions to be made of the toxic effects for many dioxins which have not been tested directly. Most studies in this area have been conducted on mammals (rats, mice and monkeys), but there are also studies on human cells.

It is generally believed that the toxic effects of dioxins are initiated at the cellular level, by the binding of the dioxin to a specific protein in the cytoplasm of the body cells, the aryl hydrocarbon receptor (AhR). The binding of TCDD to the Ah receptor constitutes a first and necessary step to initiate the toxic and biochemical effects of this compound, although it is not sufficient alone to explain the full toxic effects. This mechanism of action of TCDD parallels in many ways that of the steroid hormones, which have a broad spectrum of effects throughout the body. However, dioxin and steroid hormone receptors (e.g. oestrogen, androgen, glucocorticoid, thyroid hormone, vitamin D_3 and retinoic acid receptors) do not belong to the same family.

The mechanism of dioxin action via the AhR has, until now, been demonstrated only for the induction of metabolising enzymes capable of oxidising both alien and endogenous substances, a first step in the detoxification mechanism, which can create

substances with lesser or greater potency than the original chemical. However, subsequent mechanisms of action for the critical effects, such as cancer, reproductive toxicity, neuro- or immunotoxicity are not yet fully understood.

The modes of action discussed below are the ones most relevant from a risk assessment point of view. However, it should be recognised that in the literature there are also other mechanisms of dioxin toxicity described and also mechanisms which may not yet have been identified. Exposure to other chemicals might also cause the same measured toxic effects as dioxins and dioxin-like compounds, and measured health effects in the population may result from exposure to a broader range of chemicals in the environment.

Cytochrome P450 induction: Binding of dioxin to the Ah receptor has been shown to lead to induction and hence increased production of different enzymes, such as cytochrome P450 1A1. Subsequently, the enzyme induction leads to altered metabolism of a number of environmental chemicals and naturally occurring compounds in the body, such as hormones. The induced increase in metabolism can protect the cell from potentially damaging substances, but can also greatly increase the potential damage of other chemicals, by producing more highly toxic metabolites. In addition, the metabolism of endogenous substances, like steroid hormones may be affected leading to disturbances in critical biological functions. The induction of the cytochrome P450 1A1 enzyme is frequently used as a convenient biomarker for dioxins and other dioxin-like compounds.

Sex Hormone Effects: Dioxins cause several effects on the reproductive system of both males and females. One explanation behind these effects may be that dioxins have antioestrogenic properties, i.e. they inhibit the responses of oestrogen, a significant female sex hormone. The antioestrogenic effect is mediated through the Ah receptor, either via induced metabolism of oestrogen or by interactions between the Ah and oestrogen receptor pathways.

Cancer promotion: TCDD is a multisite carcinogen in animals as well as in humans. TCDD causes liver tumours in animals at lower concentrations than any other man-made chemical. Dioxins are not genotoxic (i.e. do not initiate cancer development), but both TCDD and other dioxins are strong promoters of tumour development. TCDD interferes with several functions that probably influence the tumour promotion process, such as growth factors, hormone systems, oxidative damage, intercellular communication, cell proliferation (division and growth), apoptosis (cell death), immune surveillance and cytotoxicity (cellular toxicity). It is generally believed, but not yet proven, that the Ah-receptor is involved in carcinogenic effects caused by TCDD.

2.2 TOXIC EFFECTS

The following sections highlight the main features of dioxin toxicity with reference to both animal and human studies.

Animal studies
Dioxin-like compounds elicit a broad spectrum of responses that are specific for the age, sex, strain, and species of the animal. Among these effects are:

- liver damage (hepatotoxicity);
- damage to the thymus and lymph systems accompanied by immune system suppression;
- the formation and development of cancers (carcinogenesis);
- abnormalities in foetal development (teratogenicity);
- developmental and reproductive toxicity;
- skin defects (dermal toxicity);
- diverse effects on hormones and growth factors;
- induction of metabolising enzyme activities (which increases the risk of metabolising precursor chemicals to produce others which are more biologically active);
- decreased food consumption;
- a wasting syndrome.

The foetus and the neonatal offspring of several species have been shown to be particularly sensitive to TCDD, resulting in effects on reproduction, immune function and behaviour.

Human studies
In humans effects associated with exposure to dioxins are mainly observed after accidental and occupational exposure. A number of cancer locations, as well as total cancer, have been associated with exposure to dioxins (mostly TCDD). In addition, an increased prevalence of diabetes and increased mortality due to diabetes and cardiovascular diseases have been reported. In children exposed to dioxins and/or PCBs during gestation, effects on neurodevelopment and neurobehaviour (object learning) and effects on thyroid hormone status (important as the body's activity regulator) have been observed at exposures at or near background levels. At higher exposures, children exposed in the womb to PCBs and PCDFs, effects included skin defects, developmental delays, low birth-weight, behaviour disorders, decrease in penile length at puberty, reduced height among girls at puberty and hearing loss. It should be noted**,** however, that it is not totally clear to what extent dioxin-like compounds are responsible for these effects, when considering the complex chemical mixtures to which human individuals are exposed.

The following sections highlight the main features of dioxin toxicity important for health risk assessment, i.e. the critical effects occurring at the lowest exposure levels. The most extensive dataset on animal toxicity is available for TCDD. In the epidemiological studies humans are most often exposed to mixtures of dioxins, other dioxin-like compounds, such as planar PCB, as well as nondioxin-like compounds, which may cause similar effects.

2.2.1 Cancer

Animal studies
Several long-term animal studies have shown that TCDD is a multisite carcinogen, a carcinogen in both sexes, and in several species. Mechanistically, TCDD is a potent tumour promoter without initiating activity[1]. The sensitivity to liver tumours in rats is dependent on sex hormones. In addition, TCDD has been shown to inhibit spontaneous mammary tumour development in female rats, an effect that agrees well with TCDDs antioestrogenic (sex hormone) activities.

Human studies
For humans, several epidemiological studies on accidental and occupational exposure to dioxins and PCBs show evidence of an increased incidence of different tumours, but the low quality and/or power of the studies make them difficult to interpret. However, from the five most important cohorts (studies of defined populations, often at-risk groups with known elevated exposures), it can be concluded that the strongest evidence for the carcinogenicity of TCDD was for all cancers combined, rather than for any specific site, although this may be influenced by the size of the population groups and the number of cancers observed, and hence their statistical significance.

Excess risks have been observed for soft tissue sarcoma (malignant tumour arising from a connective tissue) and also for lung cancer, non-Hodgkin lymphoma (cancer of the lymphatic system) and digestive tract cancers. In addition, excess risks have been observed in individual cohorts for a variety of other cancer sites including multiple myeloma (malignant tumour of the bone marrow), oral cavity cancer, renal (kidney) cancer, leukaemia and breast cancer in women.

Recently, the WHO International Agency for Research on Cancer (IARC) evaluated the available data on the carcinogenicity of dioxins and concluded that TCDD is carcinogenic to humans. Other PCDDs and PCDFs were not classifiable as to their carcinogenicity to humans. However, several PCDDs, PCDFs, as well as non-*ortho* and mono-*ortho* PCBs have been shown to promote the development of early tumour stages.

2.2.2 Reproductive toxicity

Animal studies
Dioxins have been shown to cause a variety of developmental effects on the reproductive system of both male and female animals. A very low prenatal dose of TCDD affects the reproductive development of male rats. In adult age, sperm production of male offspring is decreased and their sexual behaviour both demasculated and feminised. Reproductive alterations also occur in the female offspring, involving structural malformations in the urogenital tract. Cross-fostering studies indicate that the majority of these effects are induced by exposure before birth, rather than lactation exposure after birth. Another, possibly hormone-related, effect of

[1] Carcinogenesis is believed to be a multi-event process, and comprises an initiation phase (such as DNA damage) and a promotion phase in which sequential events advance the probability of producing cancerous cells.

TCDD observed at low doses is the occurrence of endometriosis (bleeding in the abdominal cavity, causing pain and affecting reproduction) reported in rhesus monkeys. Although the antioestrogenic effects of dioxins may be a mechanism behind at least some of the reproductive effects, the mechanisms of toxicity are not yet fully understood.

Human studies
Also in humans the developing foetus and infant have been shown to be more sensitive to reproductive toxicity of dioxins than the adult. Among the children born to mothers poisoned by PCBs and PCDFs in rice oil in Taiwan (Yucheng), low birth weight, decreased penile length at puberty, reduced height among girls at puberty and hearing loss have been reported. In addition, an alteration of the sex ratio (excess female to male) was observed in children born to parents highly exposed to TCDD. These reports are from poisoning situations, with exposure magnitudes higher than background levels. However, there are studies on infants of the general population showing lower birth weight, as well as subtle effects on thyroid hormone status, neurodevelopment and the immune system.

2.2.3 Neurotoxicity

Animal studies
Developmental effects of dioxins on the behaviour of offspring have been observed in rodents and monkeys. Effects on cognitive (learning) development have also been observed in monkey offspring exposed to low levels of TCDD in the womb and via lactation. The possible mechanism through which TCDD might exert the behavioural effects observed is not yet known. However, mechanisms involving effects on dopamine (a neurotransmitter chemical) and thyroid systems have been suggested. In general, cognitive changes produced by exposure to PCBs and dioxins in animals in the womb persist throughout adulthood.

Human studies
Developmental effects on the behaviour have also been reported for infants and children. Children exposed prenatally to high levels of PCBs and PCDFs had lower mean intelligence quotients (IQ), still apparent at 8-13 years of age. In addition, developmental effects on cognitive (learning) behaviour have been reported in children whose mothers had eaten relatively large quantities of Lake Michigan fish contaminated with PCB. Slightly lower IQ was still apparent at 11 years of age. In a Dutch study, background *in utero* and lactational exposure of human infants to levels of dioxins and PCBs was related to a delay in neurodevelopment, however, this delay was not considered serious. The effects reported were primarily associated with *in utero*, rather than lactational exposure. In fact, despite the contaminants in the milk, breast fed infants were shown to have better neurobehavioural (object learning) development compared to formula fed infants. From these epidemiological studies it is not clear whether dioxins, other dioxin-like compounds, or nondioxin-like compounds were responsible for the effects.

2.2.4 Immunotoxicity

Animal studies

TCDD and related compounds produce a vast number of effects on the immune system, often at very low doses. The cellular targets of TCDD in the immune system appear to be multiple. A prominent sign of immunotoxicity in rodents exposed to TCDD is atrophy (wasting) of the thymus, the gland responsible for the production of T-lymphocytes; important mediators in the immune response. However, the ability of dioxin to affect T-lymphocytes in monkeys and the resistance of mice to influenza viruses, has been shown to occur at doses much lower than those resulting in thymic atrophy. It has been suggested that the developing embryo/foetus may be more sensitive than the adult to immunosuppression induced by TCDD.

Human studies

There are some reports on immunological effects in humans exposed to dioxins and/or PCBs. Reduced immune response and changes in differentiation pattern of T-lymphocytes were reported in people exposed to TCDD in the Times Beach incident in the US. Besides effects on immunoglobulin (recognition factors for foreign material in the bloodstream) and T-lymphocyte numbers in the adult Yucheng population, immunosuppressive effects caused by prenatal exposure to PCBs and PCDFs have been suggested in children, as they have elevated incidences of respiratory infections and otitis (inflammatory disease of the ear). Also, infants from the general population exposed to background concentrations of dioxins and PCBs, have shown changes in certain immunological parameters. In addition, high consumption of fish from the Baltic Sea (contaminated with dioxins and PCBs) has been related to an altered differentiation pattern of T-lymphocytes in adults. Moreover, the Inuit population in Arctic Quebec, which has elevated tissue levels of PCBs and dioxins, has an increased incidence of infectious disease and otitis in the first year of life, consistent with immunosuppression.

3 Derivation of Toxic Equivalency Factors (TEFs)

Dioxins occur in widely varying mixtures in the environment. This is because each source of dioxin will generate the individual congeners in different proportions, and also because the relative proportions of each congener will change with time and with transport from one environmental compartment to another through differential degradation, metabolism, uptake or elimination rates. TCDD is the only dioxin congener for which the toxicity is relatively well characterised. However, based on the concept of a common mechanism of toxicity, all dioxins are assumed to be able to cause the same toxic effects as TCDD. It has been shown that the different congeners are not equally potent, as defined by their ability to cause specific toxic effects in animals. The potential difficulty this presents, in assessing the likely effect of a particular mixture of congeners on health, has been overcome by expressing the toxic potency of each congener as a Toxic Equivalency Factor (TEF). The TEF represents the toxicity of the congener relative to 2,3,7,8-TCDD, recognised as the most potent dioxin and assigned a TEF of 1. By summing the concentrations times the TEF of all congeners in an environmental sample an overall Toxic Equivalent (TEQ) may be derived for the sample. Thus, the risk assessment and tolerable daily intake (TDI) for TCDD can be applied to the toxic equivalents, or TCDD equivalents.

A number of different TEF-schemes have been developed for PCDDs, PCDFs and PCBs. Recognising the necessity for a more consistent approach towards setting internationally agreed TEFs, the WHO-European Centre for Environment and Health (WHO-ECEH) and the International Programme on Chemical Safety (IPCS), initiated a project to create a data base containing information relevant to the setting of TEFs. Based on the available information, WHO gathered a group of experts to assess the relative potencies and to derive consensus TEFs for PCDDs, PCDFs and dioxin-like PCBs. In 1997 the WHO expert meeting derived consensus TEFs for both human and wildlife risk assessment. The recommended TEF-values are given in the Technical Annex.

To be included in a TEF-scheme a compound has to fulfil the following criteria. It must:

- show a structural relationship to the PCDDs and PCDFs;
- bind to the Ah receptor;
- elicit Ah receptor-mediated biochemical and toxic responses;
- be persistent and accumulate in the food chain.

To avoid confusion regarding the definition of the term TEF, the WHO consultation has suggested the following terms:

- TEF as a consensus order of magnitude estimate of the toxicity of a compound relative to TCDD. The TEFs have been derived using careful scientific judgement after considering all available scientific data;
- REP (relative potency) as a potency value relative to TCDD obtained in a single *in*

vivo or *in vitro* study.

The TEFs were primarily derived from *in vivo* toxicity data, which were given more weight than *in vitro* and/or quantitative structure-activity relationship (QSAR) data. For *in vivo* toxicity data long-term exposure were prioritised. It should be noted that, while the critical effects of dioxin occur after exposure during development, in very few, if any studies useful for setting TEFs, the animals have been exposed during that period. In addition, TEFs are in many cases based on nonadverse effects, such as enzyme induction, rather than being based on the specific toxic effects. It is assumed that the TEFs for the critical toxic effects are similar to those based on nonadverse effects.

The TEF concept assumes a model of dose additivity. There has been much discussion about possible interactions between and among individual congeners in complex mixtures. Based on receptor theory, the proposed mechanism of action of Ah receptor-active compounds and a limited number of validation studies using mixtures, an additive model for the prediction of TEQs still seems most plausible, in spite of some observed nonadditive interactions. It is unlikely that the use of additivity in the TEF concept will result in a great deal of error in predicting the concentrations of TEQs due to synergism or antagonism.

The non-*ortho* and mono-*ortho* PCBs also elicit Ah receptor-mediated responses. As a consequence, TEFs have been assigned to these PCBs as well. From a risk assessment point of view this approach is relevant, since most environmental matrices contain PCDDs, PCDFs, and PCBs. In fact, in some environmental samples, the overall contribution of PCBs to TEQs exceeds that of the PCDDs and PCDFs.

In spite of uncertainties, such as nonadditive interactions, differences in shape of the dose-response curve, and species responsiveness, it has been concluded that the TEF concept is still the most plausible and feasible approach for risk assessment of halogenated aromatic hydrocarbons with dioxin-like properties.

4. Health Risk Assessment

The health risk assessment of dioxins involves:

- the identification of the critical effect, i.e. the most sensitive effect, of the exposure;
- the assessment of the dose-response relationship for this effect, including the identification of a no observed adverse effect level (NOAEL); and
- the estimation of a tolerable daily intake (TDI).

In this section different approaches to risk assessment of dioxins, as well as estimation of TDI, are reviewed and discussed. In addition, the major gaps in knowledge needed to improve the risk assessment are listed.

4.1 HEALTH RISK ASSESSMENT AND TOLERABLE DAILY INTAKE (TDI)

Different countries and organisations have used various approaches to the health risk assessment of dioxins. Until the beginning of the 1990s cancer was considered the critical effect (i.e. the most sensitive effect) and all risk assessments were based upon the same cancer study in rats. However, different approaches for the quantitative assessment of the risk to humans were applied, resulting in various estimates of the tolerably daily intake (TDI). In the early 1990s reports appeared in the literature showing the high sensitivity of the foetus and newborn young to dioxins. In experiments with exposure to dioxin during gestation and/or lactation, behavioural, immune system and reproductive effects were observed. The two most recent health risk assessments, carried out by the Health Council of the Netherlands in 1996 and WHO in 1998, are based on developmental effects initiated during gestation and/or lactation. The risk assessments presented below are summarised in Table 1.

Table 1. National and International Risk Assessments of Dioxins.

	Year	**TDI**	**Method**	**Effects (species)**
US EPA	1985	0.006 pg TCDD/kg/day	linearised multi-stage	Cancer (rat).
Nordic Council of Ministers	1988	5 pg N-TEQ/kg/day	UF 200	Cancer (rat).
WHO	1990	10 pg I-TEQ/kg/day	UF 100	Reproductive and immune effects (rat, monkey).
Health Council of The Netherlands	1996	1 pg I-TEQ/kg/day	UF 100	Cognitive development, endometriosis (monkey).
WHO	1998	1-4* pg WHO-TEQ/kg/day	UF 10	Developmental effects (rat, monkey).

UF uncertainty factor by which estimated NOAEL or LOAEL is divided to give TDI

* Calculated on body burden (not dose)

4.1.1 US Environmental Protection Agency (EPA) 1985

In 1985 the US EPA published a health risk assessment of dioxins. Dioxin (i.e. TCDD) was regulated as a carcinogen and the risk was extrapolated from a cancer study on rats by using a linearised multi-stage model. This approach was an EPA default position for carcinogens and resulted in an upper bound estimate of an excess of one in a million cancer risk from exposure to 6 fg/kg/d (corresponding to a tolerably daily intake of 0.006 pg TCDD per kilogram body weight).

In a reassessment which has yet to be finalised, the linearised multi-stage model is still used. US EPA justifies this approach by considering evidence supporting linearity in the low dose region of the experimental range for a number of dioxin-mediated responses. The Agency has not regulated dioxin based on its non-cancer effects, believing that the use of the linear multi-stage model for carcinogenesis would be protective for non-cancer effects as well.

4.1.2 Nordic 1988

In 1988, The Institute of Environmental Medicine in Sweden performed a risk assessment of dioxins at the request of the Nordic Council of Ministers. Based on animal studies, the critical effects in the low dose range were identified as cancer, reproductive and immunological effects. The quantitative risk assessment was based on the carcinogenic and reproductive effects with no/lowest observed adverse effect levels (NOAELs/LOAELs) at 1000 pg TCDD/kg body weight per day. The expert group considered the safety factor approach as relevant, taking into account available information regarding the probable underlying mechanism for TCDDs carcinogenic effect; i.e. that TCDD is a tumour promoter without genotoxic potential. Due to the lack of a reliable NOAEL a safety factor of 2 was chosen, besides the usual factor of 10 for interspecies variability and 10 for intraspecies variability. The calculations resulted in a TDI of 5 pg/kg body weight.

The TDI for TCDD was extended to cover other PCDDs and PCDFs through the recommendation of a Nordic TEF-scheme.

Infants, populations with high consumption of fish, and certain occupationally exposed populations were identified as possible risk groups, due to high exposure to dioxins.

4.1.3 WHO 1990

In 1990, WHO/EURO gathered international experts in the field of risk assessment of dioxins. It was concluded that TCDD is carcinogenic in animals but the evidence in humans was inconclusive. Since TCDD was considered to be nongenotoxic and act as a promoter-carcinogen, the consultation decided to establish a TDI based on general toxicological effects. For reproductive effects and immunotoxicity tested in various animal species, a no adverse effect level of 1000 pg/kg body weight per day was identified. By using kinetic data this level was shown to be equivalent to a dose of 100 pg/kg body weight per day in humans. Because of the poor data base on reproductive

effects in humans, an uncertainty factor of 10 was employed and thus a TDI of 10 pg TCDD/kg body weight was recommended. The consultation further recommended that the international toxicity equivalency factors (I-TEFs) should be used as an interim approach for risk management purposes, until adequate data for PCDD and PCDF congeners other than TCDD were available.

In the report from the WHO consultation it was emphasised that the TDI of 10 pg TCDD per kg body weight for the general population should not be applied to infants who are breastfed, since the TDI concept for these substances is based on a lifetime intake. However, it was stressed that, whenever possible, exposure to these compounds must be minimised in order to reduce the accumulation of PCDDs and PCDFs in breastfed infants. In addition, lactating mothers should not intentionally try to lose weight because PCDDs and PCDFs might be mobilised from fat stores during excessive weight reduction and transferred to the infant via breast milk.

4.1.4 The Netherlands 1996

In 1996, The Health Council of The Netherlands presented the first health risk assessment of dioxins, which was based on developmental effects. The Committee on Risk Evaluation of Substances arrived at its proposed health-based recommended exposure limit in the following way. Exposure to dioxin-like compounds at low dose levels does not cause cancer, but at these intake levels there may be other adverse effects. A great deal of research on the carcinogenic properties and effects on reproduction and prenatal and postnatal development show that developmental effects are the first to be observed as a result of increased exposure. For instance, changes have been observed in the cognitive development of Rhesus monkeys when the mother was exposed to approximately 100 pg TCDD per kg body weight per day or more. The mothers developed endometriosis. In another study changes in the white blood cells of Marmoset monkeys were observed at a similar level of exposure. The Committee took 100 pg/kg body weight/day to be the lowest level at which adverse effects had been observed. In order to derive a recommended level for humans from the reported animal studies, the Committee made use of extrapolation and safety factors. Using dose-response ratios for effects on rats in the lower intake range, it derived an extrapolation factor from lowest observed adverse effect level (LOAEL) to no adverse effect level (NAEL) of 2 for experimental animals. The Committee selected a factor of 5 for extrapolation from monkey to man. Differences in sensitivity between humans (intraspecies variation) were accounted for by applying the usual safety factor of 10. This reasoning leads to a figure of 1 pg TCDD per kg body weight per day, as a public health-based exposure limit for humans.

The Committee supported the use of the TEF-scheme to assess the health risk of PCDDs, PCDFs and dioxin-like PCBs.

According to the Committee, the best way to reduce the exposure of infants is to reduce the lifetime exposure of mothers, in fact the exposure of the whole population. Limitation of breast-feeding was not considered to be the right way. Since breast-feeding per se has a positive effect on the development of infants there was seen to be no reason to limit the freedom of parents to chose between breast-feeding and formula feeding for their infant. The Committee deemed that the health risk assessment

constituted an argument for further reducing existing concentrations, which result largely from human activities.

4.1.5 WHO 1998

In 1998 WHO-ECEH and IPCS jointly organised a consultation on the assessment of the health risk of dioxins and a reevaluation of the TDI. The consultation made a thorough, scientific evaluation of all available data. The risk assessment was based on LOAELs for the most sensitive adverse responses reported in experimental animals:

- decreased sperm count in offspring of rats;
- immune suppression in offspring of rats;
- increased genital malformations in offspring of rats;
- neurobehavioural (object learning) effects in offspring of monkeys;
- endometriosis in monkeys.

The LOAELs for these effects were associated with body burdens from which a range of estimated long-term human daily intakes of 14-37 pg TCDD/kg body weight was calculated.

In order to arrive at a TDI based on Toxic Equivalents (TEQs), the use of uncertainty factors had to be addressed. Since body burdens have been used to scale doses across species, the consultation concluded that the use of an uncertainty factor to account for interspecies differences in toxicokinetics was not required. However, the estimated human intake was based on LOAELs and not on NOAELs. In addition the consultation noted that, although for many parameters humans might be less sensitive than animals, still uncertainty remains regarding animal to human susceptibilities. Furthermore, differences exist in the half-lives of elimination for the different components of a TEQ mixture. To account for all these uncertainties, a composite uncertainty factor of 10 was recommended.

Based on the range of estimated human daily intakes for the most sensitive responses in animal studies, and applying this uncertainty factor, a TDI range of 1-4 pg TEQs/kg body weight was established.

The consultation recognised that subtle effects might already be occurring in the general population in developed countries at current background levels of exposure to dioxins and dioxin-like compounds. It therefore recommended that every effort should be made to reduce exposure to the lower end of this range.

Breast-fed infants are exposed to higher intakes of these compounds on a body weight basis, although for a small proportion of their lifespan. However, the consultation noted that in studies of infants, breast-feeding was associated with beneficial effects, in spite of the contaminants present. The subtle effects noted in the studies were found to be associated with transplacental, rather than lactational, exposure. Thus, the current evidence does not support an alteration of WHO recommendations that promote and support breast-feeding.

4.1.6 Discussion

The risk assessments of dioxins reviewed here use different approaches and establish different TDIs. The risk assessment of US EPA is unique in that it assumes a linear dose-response relationship for dioxin-induced cancer, which is usually only assumed

for carcinogens which damage the genetic material (DNA). The one in a million cancer risk was calculated for an exposure of 0.006 pg TCDD per kg body weight per day (corresponding to a TDI). This level lies about three orders of magnitude below the currently estimated background exposure of TEQs.

All other risk assessments used the uncertainty, or safety factor approach. Depending on the choices of critical effect and uncertainty factors, the recommended TDIs were in the range of 1-10 pg TEQ per kg body weight. These assessments supported the use of the TEF-scheme in risk assessment and risk management of PCDDs, PCDFs and, more recently, PCBs.

The WHO risk assessment performed in 1998 is the most recent risk assessment. It is of high quality due to the broad range of highly qualified international experts participating. In the WHO risk assessment all available new data on developmental effects of dioxins were evaluated. In addition, dose extrapolation from animals to humans was performed on a body burden basis, which is more toxicologically relevant than using external dose. The WHO risk assessment was based on the most recent knowledge regarding critical effects, dose-response relationships and quantitative risk extrapolation.

4.2 MAJOR GAPS IN KNOWLEDGE

Although there is a quite good basis for health risk assessment of TCDD there are still some important gaps in knowledge, especially for dioxins other than TCDD. Particular gaps in understanding are:

- dose-response relationships including no effect levels for the developmental effects in animals;
- a more reliable and complete mechanistic understanding and support for the applicability of the TEF concept to the critical effects, i.e. developmental effects, of PCDD, PCDF and PCB exposure;
- epidemiological follow-up on reproductive, neurobehavioural, immune system effects, as well as cancer in children exposed in the womb, to dioxin-like compounds. These studies should include exposure analysis in order to describe the dose-response relationships of the effects.

5. Conclusions

Work on the molecular and cellular effects of dioxins to date suggests that the way in which they act is broadly the same. This is important, because it allows assumptions to be made of the effects for many dioxins which have not been tested toxicologically. It is generally believed that the toxic effects of dioxins are initiated by the binding of the dioxin to the intracellular aryl hydrocarbon receptor (AhR). This binding leads to a subsequent regulation of gene expression. This mechanism of action of TCDD parallels in many ways that of the steroid hormones.

Dioxin-like compounds elicit a broad spectrum of responses in **experimental animals**. Among these effects are:

- liver damage (hepatoxicity);
- suppression of the immune system (immunotoxicity);
- formation and development of cancers (carcinogenesis);
- abnormalities in foetal development (teratogenicity);
- developmental and reproductive toxicity;
- skin defects (dermal toxicity);
- diverse effects on hormones and growth factors;
- and induction of metabolising enzyme activities (which increases the risk of metabolising precursor chemicals to produce others which are more biologically active).

Cancer was for long considered as the critical effect, i.e. the most sensitive effect, of dioxin exposure. However, in recent years, the foetus and newborn offspring of several species have been shown to be particularly sensitive to TCDD, resulting in effects on reproduction, immune function and behaviour.

In humans effects associated with exposure to dioxins are mainly observed in accidental and occupational exposure situations. A number of cancer locations, as well as total cancer, have been associated with exposure to dioxins (mostly TCDD). In addition, an increased prevalence of diabetes and increased mortality due to diabetes and cardiovascular diseases have been reported. In children exposed to dioxins and/or PCBs in the womb, effects on neurodevelopment and neurobehaviour and effects on thyroid hormone status have been observed at exposures at or near background levels. At higher exposures, children exposed transplacentally to PCBs and PCDFs show skin defects, developmental delays, low birth-weight, behaviour disorders, decrease in penile length at puberty, reduced height among girls at puberty and hearing loss. It is not totally clear to what extent dioxin-like compounds are responsible for these effects, when considering the complex chemical mixtures to which human individuals are exposed.

TCDD is the only dioxin congener for which the toxicity is relatively well characterised. However, based on the concept of a common mechanism of toxicity, all dioxins are assumed to be able to cause the same toxic effects as TCDD. The congeners are not equally potent, but the potential difficulty this presents, in assessing

the likely effect of a particular mixture of congeners on health, has been overcome by expressing the toxic potency of each congener as a Toxic Equivalency Factor (TEF). The Toxic Equivalent (TEQ) is the sum of the concentration times the TEF for all individual congeners of a sample. Recognising the necessity for a consistent approach towards setting internationally agreed TEFs, the WHO-European Centre for Environment and Health (WHO-ECEH) and the International Programme on Chemical Safety (IPCS), organised a consultation in order to assess the relative potencies of PCDDs, PCDFs and dioxin-like PCBs. In 1997 the WHO expert meeting derived consensus TEFs for both human and wildlife risk assessment. In spite of uncertainties, it was concluded that the TEF concept is still the most plausible and feasible approach for risk assessment of halogenated aromatic hydrocarbons with dioxin-like properties.

All risk assessments reviewed here, except the US EPA risk assessment, use the uncertainty, or safety, factor approach. Depending on the choices of critical effect and uncertainty factors, the recommended TDIs were in the range of 1-10 pg TCDD per kg body weight. These risk assessments supported the use of the TEF-scheme in risk assessment and risk management of PCDDs, PCDFs and PCBs. The WHO risk assessment from 1998 was based on the most up-to-date information and knowledge regarding critical effects, dose-response relationships and quantitative risk extrapolation. A tolerable daily intake of 1-4 pg TEQ per kg body weight was recommended.

6. Recommendations

It was recognised by the WHO expert group that subtle effects might already be occurring in the general population in developed countries at current background levels of exposure to dioxins and dioxin-like compounds. It is therefore of major importance to further reduce discharge of all dioxin-like compounds to the environment. Due to the high persistency of the dioxin-like compounds, the concentrations in the environment, as well as in food, will only decrease slowly.

With this in mind the following priority actions are recommended in order to reduce the health risk from exposure to dioxin-like compounds across the EU:

Member States should be encouraged to:

- apply the WHO recommended TDI of 1-4 pg WHO-TEQ/kg/day;
- include both dioxins and dioxin-like PCBs in the TDI for dioxin-like compounds;
- reduce as far as possible the discharge of dioxins to the environment;
- identify highly exposed groups most likely to be at risk of damage from dioxin contamination;
- investigate the need for establishing dietary recommendations for certain foodstuffs.

Effort should be committed to reducing the uncertainty associated with the health risk assessment by carefully targeted research into:

- dose-response relationships, including no adverse effect levels for the developmental effects in animals;
- a more reliable and complete mechanistic understanding and support for the applicability of the TEF concept to the critical effects, i.e. developmental effects of PCDD, PCDF and PCB exposure;
- epidemiological follow-up on reproductive, neurobehavioural, immune system effects, as well as cancer in children exposed to dioxin-like compounds in the womb. These studies should include exposure analysis in order to describe the dose-response relationships of the effects.

Task 8 – Human Toxicology

Technical Annex

CONTENTS

A.1 Introduction

This Annex provides additional technical information, with appropriate reference to the published literature, in support of the sections covered in the Summary Report. The assumed general mechanisms of action of dioxins and dioxin-like compounds are described in greater detail, as well as the most important features of dioxin toxicity. The TEF concept, including the basis for and the limitations of the concept, are discussed and the recent TEF-values recommended by the WHO are tabulated, for completeness. The final sections provide a glossary of terms and a list of the references consulted in the course of the study.

A.2 Overview of Toxicological and Epidemiological Studies

The following sections review the current understanding of the ways in which dioxins act to damage organisms at a cellular level and at the level of the individual. The review is based upon experimental animal (i.e. mammalian) and epidemiological human studies. Dioxins cause a broad spectrum of different effects in several organs and tissues. The present review, however, is mainly focussed on the critical effects of dioxin exposure, i.e. the effects occurring at the lowest exposure. These are the effects most relevant for human risk assessment and those which might occur after background dietary exposure to dioxins in the general population.

A.2.1 MODE OF ACTION

It is generally believed that the toxic effects of dioxins are initiated by the binding of the dioxin to the intracellular aryl hydrocarbon receptor (AhR). Other modes of action described below are most probably secondary to the dioxin-AhR binding. The modes of action discussed are the ones most relevant from a risk assessment point of view. However, it should be recognised that in the literature there are also other mechanisms of dioxin toxicity described and there are probably also mechanisms not yet identified.

A.2.1.1 Aryl Hydrocarbon Receptor

Work on the molecular and cellular effects of dioxins to date suggests that the way in which they act is broadly the same. This is important, because it allows assumptions of effects for many dioxins not tested toxicologically.

It is generally believed that the root cause of most (if not all) of the effects of dioxins lies in their ability to bind to the intracellular protein, the aryl hydrocarbon (Ah) receptor. This binding leads to a subsequent regulation of gene expression. The binding of TCDD to the Ah receptor constitutes a first and necessary step, however not sufficient, to elicit the toxic effects of this compound. Several reviews have described the mechanism of AhR action (Hankinson 1995, Okey *et al*. 1994, Poellinger 1995). In the cytoplasm, the unliganded AhR is present in a latent, inactive configuration associated with the 90 kDa heat shock protein (hsp90) which prevents DNA-binding and also keeps the receptor in a conformation that can bind the ligand. Ligand binding induces the loss of hsp90 and translocation of the AhR-ligand complex to the nucleus. When heterodimerized with the Ah receptor nuclear translocator protein (Arnt), the

ligand-AhR-Arnt complex is able to bind to dioxin responsive elements on the DNA and initiate transcription of specific genes. This mechanism of action of TCDD parallels in many ways that of the steroid hormones. However, dioxin and steroid hormone receptors (e.g. oestrogen, androgen, glucocorticoid, thyroid hormone, vitamin D_3 and retinoic acid receptors) do not belong to the same family (Poellinger 1995).

The mechanism of dioxin action via the AhR has hitherto been demonstrated only for the TCDD-induced induction of the enzyme cytochrome P450 1A1. The enzyme induction leads to altered metabolism of a number of endogenous compounds, such as hormones. Generally, products of other genes expressed by dioxin are either growth-regulatory proteins or drug-metabolising enzymes (Okey *et al.* 1994). Most of the existing knowledge on the mechanism of action of TCDD-induced effects other than enzyme induction is largely correlative (Clark *et al*. 1992). A number of the effects elicited by TCDD, such as lethality, thymic atrophy, induction of cleft palate, chloracne, tumour promotion, and enzyme induction, were demonstrated to segregate with affinity forms of the AhR (Poland and Glover 1980, Poland and Knutson 1982). Mice having the responsive AhR were more sensitive to these effects. In addition, structure-activity relationships demonstrate that for dioxin-like compounds, the toxicity of individual congeners is correlated with the affinity with which the congeners bind to the AhR (Okey *et al*. 1994, Safe 1990). Most of these correlative studies concern endpoints such as enzyme induction and effects on organ weights. However, for the critical effects, such as cancer, reproductive toxicity, neuro- or immunotoxicity, the mechanism of action is not fully understood.

One proposed mechanism of action of TCDD and dioxin-like compounds in perinatal systems is that they act as endocrine disrupters or "environmental hormones". In this way, these compounds provide inter- and intracellular signals that alter growth, differentiation and function of cells in a specific manner in different tissues and cell types and at different stages of development (Lindström *et al.* 1995). Thus, there may be a common mechanistic basis for the critical effects of TCDD and other dioxin-like compounds, i.e. modifications of hormonal systems.

A.2.1.1.1 Cytochrome P450 induction

Binding of TCDD to the AhR has been demonstrated to lead to induction of the enzyme cytochrome P450 1A1. Subsequently, the enzyme induction leads to altered metabolism of a number of exogenous chemicals and endogenous compounds. Although the function of this response may be to protect the cell from potentially damaging exogenous substances (Nebert and Gonzalez 1987), it can affect the metabolism of useful substances like steroid hormones, leading to disturbances in critical biological functions. Metabolism of the exogenous substance can also greatly increase its toxic potential damage (Nebert and Jensen 1979). Induction of CYP1A is among the most sensitive effects shown after dioxin exposure in animals (van Birgelen *et al*. 1995).

The dioxin-induced cytochrome P450 1A1 enzyme activity can be used as a convenient biomarker. In the ethoxyresorufin O-deethylase (EROD) bioassay the enzyme activity is measured after exposure of cells or tissues to dioxin-like compounds (Hanberg *et al*. 1991). It should be noted, however, that the EROD assay is specific for Ah receptor binding compounds, but these include also compounds other than dioxins, such as PCBs,

polybrominated diphenyl ethers and polycyclic aromatic hydrocarbons (PAH). In addition, it must be regarded as a biomarker of exposure and not of effect since the relationship between enzyme induction and the toxic effects is still unclear.

A.2.1.1.2 Antioestrogenicity

There is considerable evidence that TCDD has antioestrogenic properties (for review see Ahlborg *et al.* 1995). This evidence includes both inhibition of the development of spontaneous mammary tumours in female rats, as well as a diverse spectrum of antioestrogenic responses in rodents and in human breast cancer cell lines. The results suggest that the antioestrogenic responses of TCDD and related compounds are mediated through the Ah receptor. Although the induced metabolism of 17-estradiol by dioxin-like compounds may cause some of the antioestrogenic effects of TCDD at relatively high doses, the critical effects are probably due to interactions between the Ah and oestrogen receptor pathways (Safe *et al.* 1991, White and Gasiewicz 1993).

A.2.1.1.3 Cancer promotion

TCDD is a multisite carcinogen in animals as well as in humans. It causes liver tumours in animals at lower concentrations than any other man-made chemical (Poland *et al.* 1982). Dioxins are not genotoxic (i.e. do not initiate cancer development), but both TCDD and other dioxins are strong promoters of tumour development (IARC 1997).

TCDD causes several effects that probably influence the tumour promotion process. TCDD has been shown to exert effects on growth factors, hormone systems, cytokines and other signal transduction pathways and can therefore be considered a powerful growth dysregulator (reviewed by IARC 1997). It is generally believed, but not yet proven, that the Ah-receptor is involved in carcinogenic effects caused by TCDD (Clarke *et al.* 1991, Okey *et al.* 1994). Further suggestions of possible carcinogenic mechanisms of TCDD include oxidative damage (Wölfe and Marquardt 1996, Tritscher *et al.* 1996, Park *et al.* 1996), impaired intercellular communication (de Haan *et al.* 1995, Bager *et al.* 1997a,b), stimulation of cell proliferation and suppression of apoptosis (Buchmann *et al.* 1994, Stinchombe *et al.* 1995) and immune surveillance (reviewed by IARC 1997). Cytotoxicity may also play a role in carcinogenicity of TCDD and related compounds (IARC 1997).

A.2.2 TOXICITY

Dioxin-like compounds elicit a broad spectrum of responses that are specific for the age, sex, strain, and species of the animal studied (reviewed by Ahlborg *et al.* 1988, 1992, WHO/IPCS 1989, Peterson *et al.* 1993, Pohjanvirta and Tuomisto 1994, Poland and Knutson 1982). Among these effects are decreased food consumption, a wasting syndrome, hepatotoxicity, thymic and lymphoid involution accompanied by diverse immunosuppressive effects, carcinogenesis, teratogenicity, developmental and reproductive toxicity, dermal toxicity, diverse effects on hormones and growth factors, and induction of phase I and phase II drug-metabolising enzyme activities. The foetus and the neonatal offspring of several species have been shown to be particularly sensitive to TCDD, resulting in effects on reproduction, immune function and behaviour (Brouwer *et al.* 1995, Mably *et al.* 1992a,b, Neubert *et al.* 1993, Peterson *et al.* 1993, Schantz and Bowman 1989).

In humans effects associated with exposure to dioxins are mainly observed after accidental and occupational exposure situations. A number of cancer locations as well as total cancer have been associated with exposure to dioxins (mostly TCDD; for review see IARC 1997). In addition, an increased prevalence and mortality of diabetes and increased mortality of cardiovascular diseases have been reported (Bertazzi *et al*. 1998). Among the Yusho and Yucheng adults, accidentally exposed to high levels of PCBs and PCDFs, the chronic exposure-related effects included chloracne, conjunctivitis, sebaceous cysts and inflammation, decreased nerve conduction velocity, fatigue and malaise, hyperpigmentation and hyperkeratosis, and increased mortality from non-malignant liver disease (Yu *et al.* 1997, WHO/IPCS 1989). In children exposed to dioxins and/or PCBs during gestation effects on neurodevelopment and neurobehaviour and effects on thyroid hormone status have been observed at exposures at or near background levels (Brouwer *et al*. 1995, 1998, Huisman *et al*. 1995). At higher exposures, in Yusho and Yucheng children exposed transplacentally to PCBs and PCDFs, effects included ectodermal defects, global persistent developmental delays, low birth-weight, mild persistent behaviour disorders, decrease in penile length at puberty, reduced hight among girls at puberty and hearing loss (Guo *et al*. 1995, WHO/IPCS 1989). It should be noted, however, that it is not totally clear to what extent dioxin-like compounds are responsible for these effects when considering the complex mixtures that human individuals are exposed to.

The following sections highlight the main features of dioxin toxicity important for health risk assessment, i.e. the critical effects occurring at the lowest exposure levels. The most extensive dataset on animal toxicity is available for TCDD. In the epidemiological studies humans are most often exposed to mixtures of dioxins, other dioxin-like compounds, such as planar PCB, as well as nondioxin-like compounds.

A.2.2.1 Cancer

Several long-term studies have shown that TCDD is a multisite carcinogen, a carcinogen in both sexes, and in several species (reviewed by Lucier *et al.* 1993). In rats, mice and hamsters, TCDD has been shown to cause tumours in the liver, thyroid, lymphoid system, skin, lung, tongue, hard palate and nasal turbinates (reviewed by IARC 1997). Mechanistically, TCDD is a potent tumour promoter without initiating activity (Pitot *et al*. 1980). TCDD-induced hepatic tumours in rats are primarily found in females (Kociba *et al.* 1978), and the sensitivity to the tumours can be modulated by hormones (Lucier *et al.* 1991). Thus, interactions of TCDD with hormonally mediated events seem to be a critical component of the carcinogenic mechanisms of TCDD (Clark *et al.* 1992, Lucier *et al.* 1993). In addition, TCDD has been shown to inhibit spontaneous mammary tumour development in female rats (Kociba *et al.* 1978), an effect which agrees well with TCDDs antioestrogenic activities (reviewed by Ahlborg *et al.* 1995, Safe 1995).

Although several epidemiological studies on accidental and occupational exposure to dioxins and PCBs show relations to an increased incidence of different tumours, the low quality and/or power of the studies make them difficult to interpret. In the evaluation by IARC in 1997, a few studies were identified as the most important studies for the evaluation of the carcinogenicity of TCDD. These were four cohort studies of herbicide producers and one cohort of residents in a contaminated area from Seveso, Italy. Overall, the strongest evidence for the carcinogenicity of TCDD was for all cancers combined, rather than for any specific

site. In most of these studies excess risks were observed for soft tissue sarcoma and also for lung cancer, non-Hodgkin lymphoma and digestive tract cancers. Significant excess risks were observed in individual cohorts for a variety of other cancer sites including multiple myeloma, oral cavity cancer, kidney cancer, leukaemia and breast cancer in women. Although there is still a limited follow-up time period, there has already been observed an increased cancer mortality for cancer forms such as rectal cancer, leukaemia and multiple myeloma 15 years after the industrial accident with TCDD in Seveso (Bertazzi *et al*. 1998). Results from a recent study on Swedish fishermen's wives support an association between exposure to a mixture of persistent organochlorine compounds through fish consumption and an increased risk for breast cancer (Rylander and Hagmar 1995).

Recently, the WHO International Agency for Research on Cancer evaluated the available data on the carcinogenicity of dioxins (IARC 1997). They concluded that TCDD is carcinogenic to humans (Group 1). This conclusion was based on the following supporting evidence:

- TCDD is a multisite carcinogen in experimental animals that has been shown by several lines of evidence to act through a mechanism involving the Ah receptor;
- this receptor is highly conserved in an evolutionary sense and functions the same way in humans as in experimental animals;
- tissue concentrations are similar both in heavily exposed human populations in which an increased overall cancer risk was observed and in rats exposed to carcinogenic dosage regimens in bioassays.

Other PCDDs and PCDFs were not classifiable as to their carcinogenicity to humans (Group 3). However, several PCDDs, PCDFs, as well as non-*ortho* and mono-*ortho* PCBs can promote early stages of tumours in combination with an initiating compound-(Haag-Grönlund *et al*. 1997a,b, Hemming *et al*. 1993, NTP 1980, Schrenk *et al*. 1993, Waern *et al*. 1991).

A.2.2.2 Reproductive toxicity

Dioxins have been shown to cause a variety of developmental effects on the reproductive system of both male and female animals. A very low prenatal dose of TCDD has been shown to affect the male rat reproductive development (reviewed by Peterson *et al*. 1993). In adult age, spermatogenesis of male offspring was decreased and their sexual behaviour was both demasculinized and feminized (Mably *et al*. 1992a,b). Reproductive alterations have also been reported in the female offspring, involving structural malformations in the urogenital tract (Birnbaum 1995a). Cross-fostering studies indicate that the majority of these effects are induced by exposure before birth. Another, possibly hormone-related, effect of TCDD at low doses is the occurrence of endometriosis reported in rhesus monkeys (Rier *et al*. 1993). The antioestrogenic properties shown for TCDD and other dioxins might be involved in the mechanistic basis for the reproductive effects. However, the mechanism(s) behind the effects are not yet fully understood.

Also in humans the developing foetus and infant have been shown to be more sensitive to reproductive toxicity of dioxins than the adult. Among the children born to mothers poisoned by PCBs and PCDFs in rice oil in Taiwan (Yucheng), low birth weight, decreased penile length at puberty, reduced height among girls at puberty and hearing loss have been reported (Guo *et al*. 1995). In addition, an alteration of the sex ratio (excess female to male) was observed in children born to parents highly exposed to TCDD (Mocarelli *et al*. 1996). These

reports are from poisoning situations with exposure magnitudes higher than background exposure levels. However, there are studies on infants of the general population showing lower birth weight, as well as subtle effects on thyroid hormone status, neurodevelopment and the immune system (Brouwer *et al*. 1995, 1998, Huisman *et al*. 1995, Rylander *et al*. 1998).

A.2.2.3 Neurotoxicity

Developmental effects of dioxins on the behaviour of the offspring have been observed in rodents and monkeys (for review see Brouwer *et al*. 1995). Effects on cognitive development have also been observed in monkey offspring exposed *in utero* and via lactation to low levels of TCDD (Levin *et al*. 1988, Schantz and Bowman 1989, Schantz *et al*. 1991). The possible mechanism through which TCDD might exert the behavioural effects observed is not yet known. However, mechanisms involving effects on dopamine and thyroid systems have been suggested. In general, cognitive changes produced by *in utero* exposure to PCBs and dioxins in animals persist throughout adulthood.

Developmental effects on the behaviour have also been reported for infants and children. Prenatally exposed Yucheng children had lower mean intelligence quotients (IQ), still apparent at 8-13 years of age (Guo *et al*. 1995). The developmental effects observed in Yucheng children were caused by accidentally high exposure situations to PCBs and PCDFs. However, these reports show which effects can be expected in humans exposed to high enough levels of PCBs and dioxin-like compounds. In addition, developmental effects on cognitive behaviour have been reported in children whose mothers had eaten relatively large quantities of Lake Michigan fish, contaminated with PCB. Prenatal PCB exposure was associated with slightly lower IQ, still apparent at 11 years of age (Jacobson *et al*. 1990, Jacobson and Jacobson 1996). In a Dutch study of infants of the general population, the psychomotor scale was negatively correlated with the prenatal PCB exposure at 3, but not at 7 months of age (Koopman-Esseboom *et al*. 1996). From this study it was concluded that background *in utero* and lactational exposure of human infants to levels of dioxins and PCBs in the Netherlands is not related to a serious delay in neurodevelopment. However, levels of PCB and TCDD equivalents (TEQ) in mother's milk were negatively correlated with neonatal neurological optimality. A higher percentage of hypotonia was observed in infants exposed to higher levels of planar PCBs in breast milk (Huisman *et al*. 1995). Despite the much larger quantities of dioxin and/or PCBs transferred to the infant postnatally via breast-feeding, effects were primarily associated with *in utero*, rather than lactational exposure. Breast fed infants in The Netherlands were shown to have better neurobehavioural development compared to formula fed infants. Within the group of breast fed infants, however, those with higher exposure within the cohort to total TEQs tended to have poorer neurobehavioural test results compared to those with lower exposure. In follow-up studies on the children at 18 and 42 months of age the relationship between PCB and/or dioxin exposure and neurological effects were less clear and not found, respectively (Huisman *et al*. 1995, Lanting *et al*. 1998). From these epidemiological studies it is not clear whether dioxins, other dioxin-like compounds, or nondioxin-like compounds cause the effects.

A.2.2.4 Immunotoxicity

TCDD and related compounds produce a vast number of effects on the immune system, often at very low doses. Both the non-specific and specific, humoral and cell-mediated immune responses are suppressed. Also resistance to infectious challenges is decreased (reviewed by

Holsapple *et al*. 1991). Thus, the cellular targets of TCDD appear to be multiple. A prominent sign of immunotoxicity in rodents exposed to TCDD is atrophy of the thymus. However, the ability of dioxin to affect for example T-lymphocyte subtype pattern in marmoset monkeys and the resistance of mice to influenza viruses, has been shown to occur at doses much lower than those resulting in thymic atrophy. It has been suggested that the developing embryo/foetus may be more sensitive than the adult to immunosuppression induced by TCDD and the effects on the immune system seem to belong to the most sensitive variables affected by TCDD (Birnbaum 1995b, Neubert *et al*. 1993). The relevance for man of subtle effects, such as modifications in the pattern of T-lymphocyte surface receptors is largely unknown. Nevertheless, such changes represent clear-cut biological effects induced by TCDD.

There are some reports on immunological effects in humans exposed to dioxins and/or PCBs. Indications of reduced immune response and changes in differentiation pattern of T-lymphocytes were reported in people exposed to TCDD in the Times Beach incident in the US (reviewed by Holsapple *et al*. 1991). Besides effects on immunoglobulin and T-lymphocyte numbers in the adult Yucheng population, immunosuppressive effects caused by prenatal exposure to PCB/PCDF have been suggested in children as they have elevated incidences of respiratory infections and otitis (Rogan *et al*. 1988). In recent years, some studies have been performed also on human populations not accidentally or occupationally exposed to dioxins and PCBs. In a Dutch study of breast-fed and bottle-fed infants from the general population changes in certain immunological parameters were related to exposure to dioxins and/or PCBs (Weisglas-Kuperus *et al*. 1995). Decreases in the number of monocytes and granulocytes and an increase in the number of cytotoxic T-cells were correlated to TEQ/PCB-levels in breast milk and plasma, respectively. In addition, high consumption of fish from the Baltic Sea (contaminated with dioxins and PCBs) has been related to an altered differentiation pattern of T-lymphocytes in adults (Hagmar *et al*. 1995). Moreover, the Inuit population in Arctic Quebec, which has elevated tissue levels of PCBs and dioxins, has a 20-fold higher incidence of infectious disease and otitis in the first year of life than individuals living in the southern Quebec (Birnbaum 1995b).

A.2.3 DERIVATION OF TOXIC EQUIVALENCY FACTORS (TEFS)

The basis for the toxic equivalency factor (TEF) concept is that several PCDDs and PCDFs, as well as some PCBs have been shown to exert a number of common toxic responses similar to those observed for TCDD. There is strong evidence suggesting a common mechanism of action of TCDD and related compounds, based on the binding of these compounds to the Ah-receptor. Due to the fact that dioxin-like compounds normally exist in environmental and biological samples as complex mixtures of congeners, the concept of toxic equivalents (TEQs) has been introduced to simplify risk assessment and regulatory control (for review see van den Berg *et al*. 1998). In applying this concept, relative toxicities of dioxin-like compounds in relation to TCDD, i.e. TEFs, are determined based on *in vitro* and *in vivo* studies. This approach is useful, but has its limitations due to a number of simplifications.

A number of different TEF-schemes have been developed for PCDDs, PCDFs and PCBs. Recognising the necessity for a more consistent approach towards setting internationally agreed TEFs, the WHO-European Centre for Environment and Health (WHO-ECEH) and the International Programme on Chemical Safety (IPCS), initiated a project to create a data base

containing information relevant to the setting of TEFs. Based on the available information, WHO gathered a group of experts to assess the relative potencies and to derive consensus TEFs for PCDDs, PCDFs and dioxin-like PCBs (Ahlborg *et al*. 1994, van den Berg *et al*. 1998). In 1997 the WHO expert meeting derived consensus TEFs for both human and wildlife risk assessment (van den Berg *et al*. 1998). The recommended TEF-values for humans are given in Table A1.

To be included in a TEF-scheme a compound shall fulfil the following criteria. It must:

- show a structural relationship to the PCDDs and PCDFs;
- bind to the Ah receptor;
- elicit Ah receptor-mediated biochemical and toxic responses;
- be persistent and accumulate in the food chain.

To avoid the confusion regarding the definition of the term TEF, the WHO consultation has suggested the following terms:

- TEF as a consensus order of magnitude estimate of the toxicity of a compound relative to TCDD. The TEFs have been derived using careful scientific judgement after considering all available scientific data.
- REP (relative potency) as a potency value relative to TCDD obtained in a single *in vivo* or *in vitro* study.

The TEFs were primarily derived from *in vivo* toxicity data, which were given more weight than *in vitro* and/or quantitative structure-activity relationship (QSAR) data. For *in vivo* toxicity data long-term exposure were prioritised. It should be noted that while the critical effects of dioxin occur after exposure during development, in very few studies, if any useful for setting TEFs, have the animals been exposed during that period. In addition, TEFs are, in many cases, based on nonadverse effects, such as enzyme induction, rather than the specific toxic effects. It is assumed that the TEFs for the critical toxic effects are similar to those based on nonadverse effects.

The TEF concept assumes a model of dose additivity. There has been much discussion about possible interactions between and among individual congeners in complex mixtures. Based on receptor theory, the proposed mechanism of action of Ah receptor-active compounds and a limited number of validation studies using mixtures, an additive model for the prediction of TEQs still seems most plausible, in spite of the nonadditive interactions which are also observed. It is unlikely that the use of additivity in the TEF concept will result in a great deal of error in predicting the concentrations of TEQs due to synergism or antagonism.

The non-*ortho* and mono-*ortho* PCBs also elicit Ah receptor-mediated responses. As a consequence, TEFs have been assigned to these PCBs as well. From a risk assessment point of view this approach is relevant, since most environmental matrices contain PCDDs, PCDFs, and PCBs. In fact, in some environmental samples, the overall contribution of PCBs to TEQs exceeds that of the PCDDs and PCDFs.

Table A1. World Health Organization toxic equivalency factors (TEFs) for humans (van den Berg *et al.* 1998).

PCDD/PCDF congener	TEF	PCB congener	IUPAC no	TEF
2,3,7,8-TCDD	1	3,4,4',5-TCB	81	0.0001[a,b,c,d]
1,2,3,7,8-PeCDD	1	3,3',4,4'-TCB	77	0.0001
1,2,3,4,7,8-HxCDD	0.1[a]	3,3',4,4',5-PeCB	126	0.1
1,2,3,6,7,8-HxCDD	0.1[a]	3,3',4,4',5,5'-HxCB	169	0.01
1,2,3,7,8,9-HxCDD	0.1[a]	2,3,3',4,4'-PeCB	105	0.0001
1,2,3,4,6,7,8-HpCDD	0.01	2,3,4,4',5-PeCB	114	0.0005[a,c,d,e]
OCDD	0.0001[a]	2,3',4,4',5-PeCB	118	0.0001
2,3,7,8-TCDF	0.1	2',3,4,4',5-PeCB	123	0.0001[a,c,e]
1,2,3,7,8-PeCDF	0.05	2,3,3',4,4',5-HxCB	156	0.0005[c,d]
2,3,4,7,8-PeCDF	0.5	2,3,3',4,4',5'-HxCB	157	0.0005[c,d,e]
1,2,3,4,7,8-HxCDF	0.1	2,3',4,4',5,5'-HxCB	167	0.00001[a,e]
1,2,3,6,7,8-HxCDF	0.1	2,3,3',4,4',5,5'-HpCB	189	0.0001[a,c]
1,2,3,7,8,9-HxCDF	0.1[a]			
2,3,4,6,7,8-HxCDF	0.1[a]			
1,2,3,4,6,7,8-HpCDF	0.01[a]			
1,2,3,4,7,8,9-HpCDF	0.01[a]			
OCDF	0.0001[a]			

[a] Limited data set
[b] *In vitro* CYP1A induction
[c] QSAR modelling prediction from CYP1A induction (monkey or pig)
[d] Structural similarity
[e] No new data from 1993 review (Ahlborg *et al*. 1994)

A.3 Glossary

Ah receptor	Aryl hydrocarbon receptor, the receptor in animal and human cells to which a dioxin molecule binds and initiates gene transcription (also sometimes written as AhR).
Biomarker	A biological response to a chemical that gives a measure of exposure, individual sensitivity or toxic effect.
Critical effect	The most sensitive effect, i.e. the effect occurring at the lowest exposure.
Dioxin-like	A compound structurally similar to TCDD which binds to AhR and elicit qualitatively the same biochemical and toxic effects as TCDD.
ED50	Median effect dose, i.e. the dose that produces a defined effect in 50% of the population or produces 50% of maximal effect.
EROD	Ethoxyresorufin-O-deethylase. This enzyme is used as a biomarker for dioxins because its activity increases with dioxin exposure.
In vitro	Study in parts of an organism, e.g. cells.
In vivo	Study in a whole organism, i.e. animal.
LD50	Median lethal dose, i.e. the dose that kills 50% of the test population.
LOEL (LOAEL)	Lowest observed (adverse) effect level.
NOEL (NOAEL)	No observed (adverse) effect level.
PCB	Polychlorinated biphenyl.
PCDD	Polychlorinated dibenzo-*p*-dioxin.
PCDF	Polychlorinated dibenzofuran.
Seveso	Accident in 1976 in Italy where people were exposed to TCDD.
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin.
TEF	Toxic equivalency factor, relative toxicity of an individual congener to the most toxic congener, TCDD (which is assigned a TEF value of 1).
TEQ	Toxic Equivalent, the sum of concentrations of dioxin-like compounds times their individual TEF measured in a sample, representing the total dioxin-like toxicity of the mixture of congeners.
TDI	Tolerable Daily Intake, a limit below which humans are considered protected from toxic effects.
Yucheng	A mass outbreak of food poisoning in Taiwan in 1979 following ingestion of rice oil contaminated with PCB and PCDF.
Yusho	A mass outbreak of food poisoning in Japan in 1968 following ingestion of rice oil contaminated with PCB and PCDF.

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