Dioxins

Polychlorinated dibenzo-*p*-dioxins, dibenzofurans and dioxin-like polychlorinated biphenyls

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Health Council of the Netherlands:
Committee on Risk Evaluation of Substances/Dioxins

to
the Minister of Health, Welfare and Sport

the Minister of Housing, Spatial Planning and the Environment

the Minister of Agriculture, Nature Management and Fisheries

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Background

The Core Programme on Priority Substances run by the Ministry of Housing, Spatial Planning and the Environment (VROM) is an essential element of government policy in dealing with the 'most hazardous substances'. The aim of the policy is to limit the risk posed by these substances to public health and the structure and functions of ecosystems, at least to a maximum permissible level set by the government, and if possible to the 'negligible' level.

The Minister of VROM has requested since 1985 that exposure limits proposed by the National Institute for Public Health and Environmental Protection (RIVM) be assessed by the Health Council. In the case of dioxin-like chemicals, the President of the Health Council of the Netherlands received a joint request to this effect from the Ministers of VROM and of Health, Welfare and Sport. He placed the request before the Committee on Risk Evaluation of Substances, which was expanded for the study of this question to include experts on the properties and effect of dioxins. In accordance with the Ministers' request, the Committee also studied the relevant scientific literature which had appeared after the publication of the RIVM Criteria Document on Dioxins in 1992.

The question whether dioxin-like substances have carcinogenic properties was investigated by the Health Council's Committee on Carcinogenic Risk Assessment, whose findings are included in the present report.

Effects and recommended exposure limits

Dioxin-like substances are frequently present in the environment in the form of mixtures of many polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs) and the dioxin-like polychlorinated biphenyls (PCBs). Exposure to these compounds may have various adverse health effects, depending on the dose. A great deal of research has been done on the carcinogenic properties of these substances and their effects on reproduction and prenatal and postnatal development. These studies show that developmental effects are the first to be observed as a result of increased exposure, especially in the case of young infants. If exposure increases any further, the promotion of cancer can - after a certain level - no longer be excluded.

The toxic equivalent of a mixture

The effect of the various substances is rather diverse, but it is certain that the many dioxin-like substances act in a similar way on body cells. This finding, together with the fact that the chemicals in question frequently appear in mixtures of highly diverse composition, has led to the adoption of a group approach. One aspect of this approach, supported by the Committee, is the assignment of a toxic equivalency factor (TEF) to each dioxin-like substance. The TEF value expresses toxicity in relation to the most toxic substance of the group, 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). The toxic equivalent (TEQ_{TOTAL}) of a mixture of PCDDs, PCDFs and dioxin-like PCBs is determined by multiplying the concentration found for each individual compound by the relevant TEF value and adding up the resulting products.

The Committee deems the TEF concept to be applicable to the assessment and limitation of the risks associated with exposure to these substances by humans and by animals and ecosystems. Experimental data show that the TEFs for man, fish and birds can differ substantially. The Committee therefore believes that in estimating the ecotoxicological risk, TEFs other than those used for the toxicological evaluation for humans should be employed. The Committee supports the internationally agreed TEF values for humans; however, it believes that further research is needed before ecotoxicological TEFs can be used.

Humans

It is government policy to guarantee with reasonable certainty that an intake equal to the health-based recommended exposure limit for a particular substance will have no adverse effect on the health of the exposed persons or their offspring. Concerning

dioxin-like substances the Committee judges an ‘adverse effect’ to be each observable change which immediately or in the longer term is harmful to an organism, as well as each adaptation or response by the organism to exposure to these chemicals which may not with reasonable certainty be regarded to be harmless. On the basis of animal data, the Committee derives a recommended limit of human exposure to dioxin-like compounds of 1 picogramme of toxic equivalents per kilogramme of body weight per day. This value is lower by a factor of 10 than the figure recommended by the World Health Organisation (WHO), and the RIVM. The Committee underpins its proposal with the results of other animal studies, some of which are more recent than those used by the WHO. The Committee arrived at its proposed health-based recommended exposure limit in the following way. Exposure to dioxin-like substances at low dose levels does not cause cancer, but at these intake levels there may be other adverse effects. For instance, changes have been observed in the cognitive development of Rhesus monkeys when the mother was exposed to approximately 100 picogrammes per kilogramme of 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 takes the 100 picogramme level per kilogramme to be the lowest level at which adverse effects have 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’ to ‘no adverse effect level’ of 2 for experimental animals. Application of this figure to the above data for monkeys thus gives a ‘no adverse effect level’ of 50 picogrammes per kg. of body weight per day. The Committee then selected a factor of 5 for extrapolation from monkey to man. Monkeys appear to be closer to man with regard to the distribution of PCDDs and PCDFs between the liver and fatty tissue than, for instance, rats. Thus, the Committee does not employ the usual factor of 10 for extrapolation from rat data. Differences in sensitivity between humans (intraspecies variation) are accounted for by applying the usual safety factor of 10.

This reasoning leads to a figure of 1 picogramme per kg. of body weight per day as a public health-based exposure limit for humans.

Ecosystems

Government policy is to limit the exposure at a level at which with reasonable certainty at least 95% of the species in an ecosystem will not experience adverse effects from the substance. Ecotoxicology based recommended exposure limits are the

basis for that. The Committee uses fixed extrapolation factors in deriving these limits, taking the data on 2,3,7,8-TCDD for a sensitive species as the basis.

For 2,3,7,8-TCDD in aquatic ecosystems, the Committee has derived a recommended exposure limit of 0.1 picogrammes per litre of water and 13 000 picogrammes per 1 kilogramme of dry matter in the sediment. In establishing these concentrations it took into account the possibility of accumulation of the chemical in the food chains and the consequences of that on birds and mammals.

The Committee proposes using an ecotoxicology based recommended exposure limit of 2000 picogrammes of 2,3,7,8-TCDD per kilogramme of dry matter for terrestrial ecosystems. Here, too, it has taken accumulation in the food chain into account.

An important difference between the exposure limits in this report and those in the RIVM criteria document on Dioxins is the fact that the Committee derives ecotoxicological exposure limits for 2,3,7,8-TCDD. Only for this substance there are enough data. In some aquatic ecosystems however the exposure to dioxin-like PCBs is of greater importance.

Comparison of exposure in the Netherlands with the recommended exposure limits

Humans

Most adults in the Netherlands are exposed to approximately 2 picogrammes of toxic equivalents of dioxin-like substances per kilogramme of body weight per day. In general, it may be stated that in excess of 90% of human exposure to PCDDs, PCDFs and dioxin-like PCBs derives from the consumption of animal fats, of which 50% are contained in milk and milk products. Infants are exposed to these substances before birth as well as through the maternal milk. Their exposure through the maternal milk, expressed per kilogramme of body weight, may thus be substantially higher than that of adults.

These exposure figures may be compared to the recommended exposure limit proposed by the Committee. This leads to the conclusion that the possibility that the ingestion of dioxin-like compounds causes adverse health effects in the Dutch population cannot be excluded with reasonable certainty. The Committee believes that it is supported in this conclusion by the correspondences between the degree of prenatal and postnatal exposure to dioxin-like substances and differences in development found in studies of infants up to the age of 18 months. However, the findings of these Dutch studies gave no indication of development in infants outside what is considered to be the normal range.

According to the committee the best way to reduce the exposure of infants is to reduce the life-time exposure of mothers, in fact the exposure of the whole population. Limitation of breast-feeding is not the right way. It was already known and recent studies confirmed that breast feeding has a positive effect on the development of infants compared to formula-feeding. There is no reason to limit the freedom of parents to choose between breast-feeding and formula-feeding for their infant.

Ecosystems

The recommended exposure limit for the sediment in aquatic ecosystems is exceeded locally. Particularly in the case of predators dependent on the aquatic environment, effects can be anticipated at current levels of exposure at certain locations. The process of bioaccumulation in the aquatic ecosystem which plays a role in this cannot be predicted with accuracy because of the presence of suspended and dissolved organic material and sediment which affect the availability of PCDDs, PCDFs and dioxin-like PCBs by binding them and releasing them again. The Committee also points out that increased binding causes the availability of dioxin-like substances to organisms to decline if dioxins and sediment are in contact with each other for long periods. Owing to this ‘ageing’ phenomenon, bioavailability in nature is in most cases lower than in laboratory experiments.

For terrestrial ecosystems only TEQ values are available and not 2,3,7,8-TCCD concentrations. Therefore it is not possible for the Committee to compare concentrations in soil with the recommended ecotoxicological recommended exposure limits.

Conclusion

Given current concentrations of dioxin-like substances in the Dutch environment, the recommended levels for humans and in some cases for ecosystems are being exceeded. The Committee deems this to be a matter for concern and believes that it constitutes an argument for further reducing existing concentrations, which are caused largely by human activities.

Samenvatting, conclusies en aanbevelingen

Kader

Het Kernprogramma prioritaire stoffen van het Ministerie van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer (VROM) is onderdeel van het regeringsbeleid ten aanzien van de ‘meest milieubedreigende stoffen’. Het doel van dat beleid is het risico dat dergelijke stoffen opleveren voor de gezondheid en voor de structuur en functies van ecosystemen in elk geval te beperken tot een door de overheid gesteld maximaal ‘toelaatbaar’ niveau en zo mogelijk verder terug te dringen tot een ‘verwaarloosbaar’ niveau.

De minister van VROM vraagt sinds 1985 de Gezondheidsraad om door het Rijksinstituut voor Volksgezondheid en Milieu (RIVM) voorgestelde advieswaarden die overeenkomen met het genoemde maximaal toegestaan risiconiveau, te toetsen aan de stand der wetenschap. Voor de dioxine-achtige stoffen ontving de voorzitter van de Gezondheidsraad een dergelijk verzoek van de ministers van VROM en van Volksgezondheid, Welzijn en Sport (VWS) te zamen. Hij legde dit verzoek voor aan de Commissie ‘Risico-evaluatie van stoffen’, die ter beantwoording van de gestelde vragen werd uitgebreid met deskundigen op het gebied van de eigenschappen en de werking van dioxinen. De commissie heeft in overeenstemming met het verzoek van de bewindslieden ook de wetenschappelijke literatuur die ná publikatie van het RIVM-Basisdocument Dioxinen is verschenen (1992) in haar beschouwingen betrokken.

Over de vraag of dioxine-achtige stoffen kankerverwekkende eigenschappen hebben, heeft de Commissie 'Beoordeling carcinogeniteit van stoffen' van de Gezondheidsraad zich gebogen; de bevindingen van die commissie zijn in het voorliggende rapport verwerkt.

Effecten en advieswaarden

Dioxine-achtige stoffen zijn vrijwel altijd in het milieu aanwezig als mengsels van tal van polygechloreerde dibenzo-*p*-dioxinen (PCDD's), dibenzofuranen (PCDF's) en dioxine-achtige polychloorbifenylen (PCB's). Blootstelling aan deze verbindingen kan, afhankelijk van de dosis, op verscheidene manieren de gezondheid nadelig beïnvloeden. Veel onderzoek is verricht naar de kankerverwekkende eigenschappen van de stoffen en naar hun invloed op de voortplanting en de ontwikkeling voor en na de geboorte. Beïnvloeding van de ontwikkeling is blijkens deze onderzoekgegevens het effect dat bij toenemende blootstelling, in het bijzonder bij jonge kinderen, als eerste wordt waargenomen. Neemt de blootstelling nog verder toe dan kan, vanaf een zekere waarde, ook het teweegbrengen van kanker niet meer worden uitgesloten.

Toxisch equivalent van een mengsel

De werkzaamheid van de diverse dioxine-achtige stoffen loopt nogal uiteen, maar wel staat vast dat zij op overeenkomstige wijze op lichaamscellen aangrijpen. Deze bevinding, gevoegd bij het feit dat de stoffen veelal in mengsels met uiteenlopende samenstelling voorkomen, heeft geleid tot een groepsgewijze benadering. Onderdeel van die benadering, waarbij de commissie zich aansluit, is het toekennen van een toxische-equivalentiefactor (TEF) aan elke dioxine-achtige stof. De TEF-waarde brengt de giftigheid ten opzichte van de giftigste verbinding van de groep, 2,3,7,8-tetrachloordibenzo-*p*-dioxine (2,3,7,8-TCDD), tot uitdrukking. Het toxische equivalent (TEQ_{TOTAAL}) van een mengsel van PCDD's, PCDF's en dioxine-achtige PCB's wordt bepaald door per individuele verbinding de aangetroffen concentratie te vermenigvuldigen met de betreffende TEF-waarde en de zo verkregen produkten te sommeren.

De commissie acht het TEF-concept toepasbaar bij het beoordelen en beteugelen van de risico's verbonden aan blootstelling van de mens en van dieren en ecosystemen. Experimentele gegevens geven aan dat de TEF's voor mensen, vissen en vogels aanzienlijk kunnen verschillen. De commissie meent daarom dat bij de ecotoxicologische risicoschatting andere TEF's moeten worden gebruikt dan bij de humaan-toxicologische. Zij onderschrijft de internationaal overeengekomen TEF-waarden voor de mens. Om ecotoxicologische TEF's te kunnen gebruiken acht de commissie nader onderzoek nodig.

Mens

De overheid vindt dat een blootstelling gelijk aan de gezondheidskundige advieswaarde voor een bepaalde stof met redelijke zekerheid moet garanderen dat zich geen ongewenst effect voordoet op de gezondheid van mens of diens nageslacht. In het geval van dioxine-achtige stoffen ziet de commissie als ‘ongewenst effect’ elke waargenomen verandering die direct of op langere termijn schadelijk is voor een organisme, alsmede elke, niet met zekerheid onschadelijke aanpassing of reactie van het organisme op de blootstelling aan deze stoffen. Voor de dioxine-achtige verbindingen leidt de commissie uit proefdiergegevens een gezondheidskundige advieswaarde voor de mens af van 1 picogram toxische equivalenten per kilogram lichaamsgewicht per dag. Deze waarde is een factor 10 lager dan het door de Wereldgezondheidsorganisatie (WHO) aanbevolen en door het RIVM onderschreven getal. De commissie onderbouwt haar voorstel met de resultaten van ander, deels recenter, proefdieronderzoek dan waarop dat van de WHO berust. De commissie kwam op de volgende wijze tot de door haar voorgestelde waarde. Blootstelling aan dioxine-achtige stoffen leidt bij lage niveaus niet tot kanker. Bij die niveaus kan wel sprake zijn van andere ongewenste effecten. Zo zijn veranderingen gevonden in de cognitieve ontwikkeling van Rhesus-apen bij blootstelling van de moeder aan ongeveer 100 picogram per kilogram lichaamsgewicht per dag of meer. De moederapen ontwikkelden endometriose. In ander onderzoek nam men bij Marmoset-apen veranderingen in witte bloedcellen waar bij een overeenkomend blootstellingsniveau. De commissie beschouwt de waarde van 100 picogram per kilogram als het laagste niveau waarbij ongewenst geachte effecten zijn waargenomen.

Om uit de vermelde proefdiergegevens een gezondheidskundige advieswaarde voor de mens af te leiden, past de commissie extrapolatie- en veiligheidsfactoren toe. Uit dosis-responsrelaties voor effecten die bij ratten in het lage-dosisgebied zijn waargenomen, leidt zij een extrapolatiefactor van ‘laagst waargenomen ongewenst effect’ naar ‘geen ongewenst effect’ bij proefdieren af van 2. Toepassing van dit getal op de genoemde gegevens over apen leidt dan tot een ‘geen ongewenst effect’-niveau van 50 picogram per kilogram lichaamsgewicht per dag. De commissie kiest vervolgens een factor 5 voor de extrapolatie van aap naar mens. De aap lijkt namelijk wat betreft de verdeling van PCDD’s en PCDF’s tussen lever en vetweefsel meer op de mens dan bijvoorbeeld de rat. Daarom gebruikt de commissie hier niet de gebruikelijke factor 10 voor extrapolatie vanuit gegevens over ratten. Verschillen in gevoeligheid tussen mensen onderling (intraspeciesvariatie) brengt zij in rekening met de gebruikelijke veiligheidsfactor van 10.

Deze redenering leidt tot 1 picogram per kilogram lichaamsgewicht per dag als gezondheidskundige advieswaarde voor de mens.

Ecosystemen

Het overheidsbeleid is erop gericht de blootstelling zodanig te beperken dat met redelijke zekerheid minimaal 95% van de soorten in een ecosysteem geen schade zal ondervinden van de stof. Ecotoxicologische advieswaarden zijn hiervoor de basis. De commissie maakt bij de afleiding van ecotoxicologische advieswaarden gebruik van vaste extrapolatiefactoren, uitgaande van gegevens voor 2,3,7,8-TCDD bij een gevoelige soort.

De commissie heeft voor 2,3,7,8-TCDD in aquatische ecosystemen een ecotoxicologische advieswaarde afgeleid van 0,1 picogram per liter water en van 13 000 picogram per kilogram droge stof in het sediment. Bij het afleiden van deze waarden heeft zij rekening gehouden met de mogelijkheid van accumulatie van de stof in de voedselketens en de gevolgen daarvan voor vogels en zoogdieren.

Voor terrestrische ecosystemen stelt de commissie een ecotoxicologische advieswaarde voor van 2000 picogram 2,3,7,8,-TCDD per kilogram droge stof. Ook in dit geval heeft de commissie rekening gehouden met accumulatie in de voedselketen.

Een belangrijk verschil tussen de hier afgeleide advieswaarden voor ecosystemen en de in het Basisdocument Dioxinen voorgestelde waarden is het feit dat de commissie zich beperkt tot 2,3,7,8-TCDD. Alleen voor deze stof zijn voldoende gegevens beschikbaar. Overigens is in aquatische ecosystemen de blootstelling aan bepaalde dioxine-achtige PCB's van groter belang.

Vergelijking van de blootstelling in Nederland met de advieswaarden

Mens

De blootstelling van de meeste volwassenen in Nederland bedraagt ongeveer 2 picogram aan toxische equivalenten van dioxine-achtige stoffen per kilogram lichaamsgewicht per dag. In het algemeen kan men stellen dat meer dan 90 procent van de blootstelling van de mens aan PCDD's, PCDF's en dioxine-achtige PCB's voortvloeit uit de consumptie van dierlijke vetten, waarvan de helft uit melk en melkprodukten. Baby's worden al vóór de geboorte en via de moedermelk blootgesteld aan deze stoffen. Hun blootstelling via de moedermelk, uitgedrukt per kilogram lichaamsgewicht per dag, kan aanmerkelijk hoger zijn dan die van volwassenen.

Deze blootstellingsgegevens kan men afzetten tegen de door de commissie voorgestelde gezondheidskundige advieswaarde. Dit leidt tot de slotsom dat gezondheidseffecten binnen de Nederlandse bevolking door blootstelling aan dioxine-achtige verbindingen niet met redelijke zekerheid zijn uit te sluiten. De commissie meent in deze conclusie te worden gesteund door de verbanden tussen de

mate van pre- en postnatale blootstelling aan dioxine-achtige stoffen en verschillen in ontwikkeling die zijn gevonden bij zuigelingen tot een leeftijd van 18 maanden. Overigens was er bij deze bevindingen uit Nederlands onderzoek geen sprake van een ontwikkeling van de zuigelingen die buiten het normaal te achten bereik viel.

Volgens de commissie kan men de blootstelling van kinderen voor de geboorte en tijdens de zoogperiode het best beperken door het handhaven van een blootstellingsnorm voor de moeder, en daarmee voor de hele bevolking. Beperking van borstvoeding is naar haar mening niet de aangewezen weg. Uit Nederlands onderzoek onder zuigelingen blijkt namelijk dat borstvoeding nog altijd positief bijdraagt aan de ontwikkeling van zuigelingen in vergelijking tot het alternatief: flesvoeding. Er is geen reden de vrijheid van ouders te beperken in de keuze tussen borstvoeding en flesvoeding voor hun kind.

Ecosystemen

In aquatische ecosystemen wordt de advieswaarde voor 2,3,7,8-TCDD in waterbodems lokaal overschreden. Vooral bij predatoren die afhankelijk zijn van het aquatisch milieu, zijn effecten te verwachten bij het huidige niveau van blootstelling op bepaalde lokaties. Het proces van bio-accumulatie in het aquatisch ecosysteem dat hierbij een rol speelt, laat zich echter niet goed voorspellen vanwege de aanwezigheid van gesuspendeerde en opgeloste organische stof en van sediment dat de beschikbaarheid van PCDD's, PCDF's en dioxine-achtige PCB's beïnvloedt door deze stoffen te binden en weer vrij te geven. De commissie wijst er ook op dat de biobeschikbaarheid van dioxine-achtige stoffen afneemt na een lange contacttijd tussen deze stoffen en sediment. Door dit verschijnsel van 'ageing' is in het veld de biobeschikbaarheid vrijwel altijd lager dan bij laboratoriumexperimenten.

Voor het terrestrisch ecosysteem zijn slechts TEQ-gehalten in de bodem beschikbaar en geen concentraties 2,3,7,8-TCDD. Daardoor is het niet mogelijk een uitspraak te doen over het al dan niet lokaal overschrijden van de advieswaarden voor bodemdieren en voor vogels en zoogdieren.

Tot slot

Gegeven de huidige concentraties van dioxine-achtige stoffen in het Nederlandse milieu worden de advieswaarden, zowel die voor de mens als in een aantal gevallen die voor ecosystemen overschreden. De commissie acht dit een reden tot zorg en een argument om de concentraties, die vrijwel geheel door menselijk handelen zijn veroorzaakt, verder terug te dringen.

Chapter 1

Introduction

1.1 Policy in the Netherlands

The Core Programme on Priority Substances run by the Ministry of Housing, Spatial Planning and the Environment (VROM) is an essential element of government policy in dealing with the 'most hazardous substances' (SVS94). The aim of the policy is to limit the risk posed by these substances to public health and the structure and functions of ecosystems, at least to a maximum permissible level set by the government. The occurrence of these substances in the environment should, as far as is reasonably feasible, be kept below that level, if possible to a predetermined 'negligible' level (TK89).

The formulation of policy and the determination of the norm levels referred to above for substances of this type are preceded by experimental and desk research. The results of this research are published in criteria documents, drafted under the auspices of the National Institute for Public Health and Environmental Protection (RIVM). The Minister of VROM has requested since 1985 that exposure limits for humans and the maximum permitted concentrations for ecosystems proposed in these criteria documents be assessed by the Health Council in the light of current scientific knowledge.

1.2 The Committee's working procedure

1.2.1 The Committee on Risk Evaluation of Substances

The Health Council's Committee on Risk Evaluation of Substances was set up by the President of the Health Council on 22 May 1991. It consists of both permanent members and, for the period during which a specific substance or group of substances is being dealt with, a number of temporary members as well. The composition of the Committee that drafted the present document on dioxins is given in Annex C.

1.2.2 The inquiry

On 11 January 1995, the Minister of Health, Welfare and Sport (VWS), on behalf of the Minister of VROM as well, submitted a written request to the Health Council asking for it to give an opinion on the Criteria Document on Dioxins. In addition, the Ministers submitted the following questions to the Council:

1 Do the existing recommended exposure limits for the maximum permitted exposure of humans and ecosystems adequately deal with the possibility of secondary poisoning?
2 Does the Health Council share the view that 2,3,7,8-TCDD must be considered a non-genotoxic carcinogen?
3 What is the view of the Health Council on the significance for the risk evaluation of dioxins of possible developmental disturbances resulting from perinatal exposure? Which is the more important in this regard: prenatal exposure or postnatal exposure?
4 Does the Health Council think that a risk evaluation of dioxins and of polychlorinated biphenyls with a dioxin-like effect should be carried out in combination? If so, how can this view be given expression in the setting of norms?

1.2.3 The response

In accordance with the request made by the Ministers of VROM and VWS, the Committee has also taken into consideration scientific literature that appeared after the Criteria Document was completed. Important reports that appeared after the Criteria Document was published include, among others:

- the draft version of the EPA document 'Estimating exposure to dioxin-like compounds' (EPA94a) and the 'Health assessment document for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) and related compounds' (EPA94b)

- Dutch doctoral dissertations, and articles based upon these dissertations, on the toxic effect of polyhalogenated aromatic hydrocarbons on the development of infants (Bro94, Hui95a, Hui95b, Koo94a, Koo94b, Plu93a, Sau94, Wei95), the effects of these substances on fish-eating birds (Bos92, Bos94, Bos95) and mammals (Ros95, Swa95), the toxic and biochemical effects of PCBs in comparison with and in combination with 2,3,7,8-TCDD in rats (Bir95), and the bioavailability of PCDDs and PCDFs in the aquatic ecosystem (Loo93, Loo94a, Loo94b).

In accordance with the usual procedure for the assessment of criteria documents, the Committee on Carcinogenic Risk Assessment has devoted its energies to investigating the question: are dioxins carcinogenic and, if so, to what extent? The findings of that Committee are included in Annex A. They are endorsed by the present Committee, and have been incorporated into the present report.

The Health Council has undertaken assessments of dioxin-like substances on previous occasions. The resulting reports addressed themselves to the contamination of the maternal milk by polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) (GR85a, GR86a, GR91a). The Committee has taken these earlier studies into consideration in the preparation of the present report.

1.3 Organisation of the report

In chapter 2, the Committee makes some general remarks regarding PCDDs, PCDFs and dioxin-like PCBs. These act as a framework for the response to the Ministers' questions. In chapter 3, the Committee goes into deriving a recommended exposure limit for the exposure of human beings to dioxin-like substances. The Committee's arguments are based on laboratory animal data, and also discuss the results of the Dutch infant study. Chapter 4 addresses itself to the derivation of recommended exposure limits for terrestrial and aquatic ecosystems. The data for farm animals are also discussed. Chapter 5 contains the responses of the Committee to the questions asked by the Ministers.

Chapter **2**

General data

2.1 PCDDs, PCDFs and PCBs: chemical structure

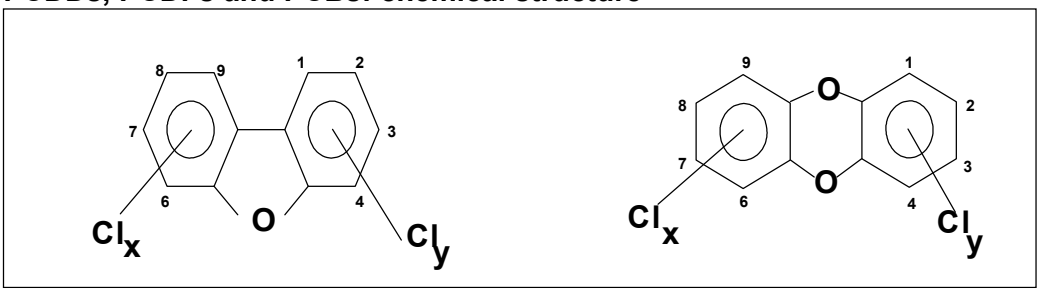

Figure 1 Structure of furans (left) and dioxins (right). x = 0 to 4, y = 0 to 4, x+y are at least 1.

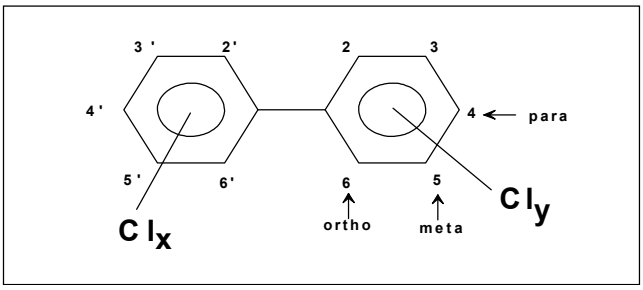

Figure 2 Structure of PCBs. x = 0 to 5, y = 0 to 5, x+y are at least 1.

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are tricyclic aromatic hydrocarbons with similar physical and chemical properties (see figure 1)*. The two classes of substances have a similar structure and are often collectively referred to simply as 'dioxins'. Specific polychlorinated biphenyls (PCBs), the so-called non-ortho-, mono-ortho- and di-ortho-congeners**, have a similar spatial structure to the dioxins (figure 2). In the opinion of the Committee, this similarity in structure (and effect, see 2.2) constitutes a reason for including a number of PCBs in the assessment of dioxins. The family of polychlorinated dibenzo-*p*-dioxins comprises 75 congeners, that of the polychlorinated dibenzofurans 135. The PCBs form a group of, in total, 209 congeners.

2.2 Toxic equivalency factors (TEFs) and additivity

Polyhalogenated aromatic hydrocarbons are almost always present in the environment in the form of mixtures of isomers*** and congeners. This phenomenon, combined with the similarity in their working mechanism, has led to these substances being approached as a group for the purpose of risk evaluation.

Man

The 2,3,7,8-tetrachloride dibenzo-*p*-dioxin (2,3,7,8-TCDD) acts as reference in assessing the toxic effect of those polyhalogenated aromatic hydrocarbons that have a similar effect. It is both the most thoroughly investigated and the most toxic of this class of compounds with a dioxin-like effect.

Only 7 of the 75 congeners of the polychlorinated dibenzo-*p*-dioxins have an effect similar to that of 2,3,7,8-TCDD. Only 10 of the 135 possible congeners of the polychlorinated benzofurans have an effect similar to that of 2,3,7,8-TCDD. Taken together, we therefore have 17 different PCDDs and PCDFs with an effect of this kind (EPA94b, Saf90).**** The capacity of the 209 PCB congeners to exert a dioxin-like effect varies; this potential is considerably greater in the case of 13 PCBs than it is in

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* There are similar groups of substances in which the chlorine atoms are replaced by bromine atoms. These substances are abbreviated to, respectively, PBDDs and PBDFs. In addition, there are similar compounds that contain both chlorine and bromine atoms.

** For the meaning of the term 'congeners', and of other terms and abbreviations, the Committee refers the reader to Annex F.

*** See Annex F.

**** The same applies to the PBDDs and the PBDFs. Regarding the occurrence and the toxicity of the brominated dioxins, little data is available. That is also the case for mixed chlorinated and brominated compounds. It is well-known that their concentrations in the environment are small compared to those of the chlorinated compounds. The Committee is leaving these substances out of consideration in the present report.

Table 1 Toxic equivalency factors (TEFs) for PCDDs and PCDFs (NATO88a,b).

designation of substance formula	IUPAC N°	$TEF_{DIOXINS}$
*PCDD*s		
2,3,7,8-TCDD	48	1
1,2,3,7,8-PeCDD	54	0.5
1,2,3,4,7,8-HxCDD	66	0.1
1,2,3,6,7,8-HxCDD	67	0.1
1,2,3,7,8,9-HxCDD	70	0.1
1,2,3,4,6,7,8-HpCDD	73	0.01
OCDD	75	0.001
other PCDDs		0
*PCDF*s		
2,3,7,8-TCDF	83	0.1
1,2,3,7,8-PeCDF	94	0.05
2,3,4,7,8-PeCDF	114	0.5
1,2,3,4,7,8-HxCDF	118	0.1
1,2,3,6,7,8-HxCDF	121	0.1
1,2,3,7,8,9-HxCDF	124	0.1
2,3,4,6,7,8-HxCDF	130	0.1
1,2,3,4,6,7,8-HpCDF	131	0.01
1,2,3,4,7,8,9-HpCDF	134	0.01
OCDF	135	0.001
other PCDFs		0

the rest (Ahl94). All PCDDs, PCDFs and PCBs with a dioxin-like effect have chlorine atoms in the 2,3,7 and 8 positions (see figures 1 and 2).

So-called TEF values have been assigned to all polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans with chlorine atoms in the 2, 3, 7 and 8 positions. These toxic equivalency factors indicate the toxic effectiveness of dioxin-like compounds compared with that of the reference compound 2,3,7,8-TCDD; the TEF of this latter compound is, by definition, 1. The TEF values for PCDDs and PCDFs have been derived from data originating from *in vitro* and *in vivo* research into acute toxicity, subchronic and chronic toxicity, and carcinogenic and teratogenic effects; they were laid down following international consultation (NATO88a, NATO88b; see table 1). The Committee is adopting these TEF values, which are referred to in the literature as I-TEF; in the text that follows they are referred to as $TEF_{DIOXINS}$. In the Committee's opinion, however, these values are not completely fixed, but need to be adjusted in conformity with new toxicological data.

During a meeting held under the auspices of the World Health Organisation (WHO) - and organised within the context of the International Programme on Chemical Safety (IPCS) - the question of whether it was also possible to lay down

internationally accepted TEF values for the dioxin-like PCBs was considered. The participants in that meeting have proposed interim TEF values (TEF^*_{PCB}s) (Ahl94; see table 2). The criteria for including a PCB in the TEF^*_{PCB} scheme were:

- the structure of the relevant PCB resembles that of PCDDs and PCDFs
- the PCB binds to the Ah receptor (see 2.3)
- the PCB causes dioxin-like biochemical and toxic effects
- the PCB is not rapidly degraded in the environment (i.e. it is persistent) and it accumulates in the food chain.

The TEF^*_{PCB}s have been derived from the results of research using laboratory animals that were administered the compound under study either repeatedly or once only, or alternatively with the help of structure-activity relationships and data from *in vitro* research. According to the participants in the WHO meeting, the use of the TEF^*_{PCB}s in the derivation of recommended exposure limits and norms offers an ample degree of public health protection.

A list examined by Safe (Saf94) contains the same PCBs as the WHO/IPCS list, but differs in some TEF values (Ahl94); see table 2, where Safe's TEF values are referred to as TEF^{**}_{PCB}. Safe asserted that the potential of a PCB is strongly dependent

Table 2 Toxic equivalency factors for the exposure of man to dioxin-like PCBs. TEF^*_{PCB}: 'interim' values proposed by WHO/IPCS (Ahl94); TEF^{**}_{PCB}: values proposed by Safe (Saf90, Saf94).

designation of substance		TEF^*_{PCB}	TEF^{**}_{PCB}
formula	IUPAC N°		
non-ortho-PCBs			
3,3',4,4'-TCB	77	0.0005	0,01
3,3',4,4',5-PeCB	126	0.1	0,1
3,3',4,4',5,5'-HxCB	169	0.01	0,05
mono-ortho-PCBs			
2,3,3',4,4',-PeCB	105	0.0001	0,001
2,3,4,4',5-PeCB	114	0.0005[a b]	0,0002
2,3',4,4',5-PeCB	118	0.0001	0,0001
2',3,4,4',5-PeCB	123	0.0001	0,0005
2,3,3',4,4',5-HxCB	156	0.0005[b]	0,0004
2,3,3',4,4',5'-HxCB	157	0.0005[b]	0,0003
2,3',4,4',5,5'-HxCB	167	0.00001[a]	0,001
2,3,3',4,4',5,5'-HpCB	189	0.0001[a]	0,001
di-ortho-PCBs			
2,2',3,3',4,4',5-HpCB	170	0.0001[a]	0,00002
2,2',3,4,4',5,5'-HpCB	180	0.00001[a]	0,00002

[a] Based on very limited data.

[b] The same TEF values are attributed to the compounds with the IUPAC numbers 114, 156, and 157 because of the similar response. This finds support in the similarity in structure.

on the species of organism concerned and on the effect that is being examined. Of the non-ortho-PCBs in table 2, the most toxic is 3,3',4,4',5-PentaCB. On the basis of a comparison of the loss in body weight, thymus atrophy, foetal thymus-lymphoid-development and enzyme induction that took place when PCB and 2,3,7,8-TCDD were administered, Safe assigned to 3,3',4,4',5-PentaCB a TEF of 0.1; this value coincides with that of the WHO/IPCS. The TEF_{PCB} values on the two lists diverge by a factor of between 2 and 100.

The Committee considers both proposals tenable in the light of the present state of scientific knowledge. In addition, it points out that the uncertainty in the exposure to PCBs is much greater than the difference in the calculated dose when using one or the other TEF_{PCB} list.

With the help of the TEF values, we can express the exposure to mixtures of PCDDs, PCDFs and dioxin-like PCBs in the form of the so-called toxic equivalency (TEQ). The TEQ value is arrived at by multiplying the concentration of each component in the mixture by the corresponding $TEF_{DIOXINS}$ or TEF_{PCB} value, and then adding together the products obtained. The starting-points in this application of the TEF concept are that the individual compounds have a similar working mechanism, and that they have an additive contribution to the toxicity of the mixture. To the latter assumption the Committee adds the following note. In relation to dioxins, specific PCBs appear to display an antagonistic or synergistic, and therefore a non-additive effect (Ahl92a, Ahl94, Ber94a, Saf90). This phenomenon is unrelated, however, to possible carcinogenic and behavioural effects that are not mediated by the Ah receptor (see 2.3; Ahl92a, Saf90).

The Committee thinks that the TEF concept is a usable and uniform instrument in the estimation of the risks associated with exposure to PCDDs, PCDFs and dioxin-like PCBs.

Ecological considerations

Experimental data on fish and birds indicate that the TEF concept can also be used to evaluate the ecotoxicological effect of dioxin-like substances (Ahl92a). The TEFs for different animals can differ considerably (Ahl94, Bos95). The Committee is therefore of the opinion that, in estimating ecotoxicological risk, separate TEFs (ECOTEFs) must be used for mammals, birds, fish and invertebrates.

2.3 Working mechanism

The working mechanism of dioxin-like compounds has been extensively studied over the past decades via biochemical and molecular biological research (Whi89). Both in laboratory animals and in humans, it seems that it is possible for 2,3,7,8-TCDD to cause effects via binding to an intracellular receptor, the 'aromatic hydrocarbon receptor' or Ah receptor (RIVM93). There seems to be general agreement within the scientific world on how this mechanism functions.

Figure 3 shows the Ah receptor mechanism schematically. After binding of the 2,3,7,8-TCDD to the Ah receptor, the so called heat shock protein is released. The Arnt protein provides for the transport to the cell nucleus (Arnt stands for 'Ah receptor nuclear translocator'). Here the Ah receptor complex can initiate a binding with so-called dioxin-responsive elements on the DNA. As a result, different genes are stimulated into expression. This then induces the production of proteins, such as the cytochrome P4501A1. The presence of this CYP1A1 protein is used as a yardstick for the dioxin-like effect of substances. In rats, the CYP1A1 protein seems to be formed even through exposure to very low doses of 2,3,7,8-TCDD, as also is the related CYP1A2 protein (Bir94a, Koh93).

From the data on 2,3,7,8-substituted PCDDs and PCDFs, it can be concluded that it is the compounds themselves and not their metabolites that set the Ah receptor mechanism in motion (Mas86, Mas88, Web82). Results of research into the binding of PCDDs and PCDFs and of their hydroxymetabolites support this conclusion (Den85, Den86). However, the metabolites referred to do display, both *in vitro* and *in vivo*, an affinity for binding with the protein complex transthyretine (which provides for the transport of thyroid hormone and vitamin A; Bro94, Lan94). For PCDDs and PCDFs, this probably has a limited significance, however, because in all animal species the hydroxylation of almost all 2,3,7,8-substituted PCDDs and PCDFs takes place extremely slowly, due to the presence of chlorine atoms in the 2, 3, 7, and 8 positions (Ber94a).

The hydroxymetabolites of PCBs can, however, cause a clear biological and possibly toxic effect in the case of both animals and man. Because this concerns PCBs with a non-dioxin-like effect, we have left the hydroxymetabolites out of consideration.* In marine mammals and in man, other conversion products of PCBs,

* These hydroxymetabolites of PCBs can remain selectively present in the blood plasma of rats, mice, seals and humans (Ber94c), and can accumulate in the foetus (Mor95) by binding to the plasma protein transthyretine (Lan94). Their presence can lead to the disturbance of communications between cells and of the concentrations of thyroid hormone and vitamin A in the blood. The metabolites display an oestrogenic and anti-oestrogenic effect, and can contribute to disturbances in functional development (Bro94). The Ah receptor mechanism supposedly plays no part in the toxic

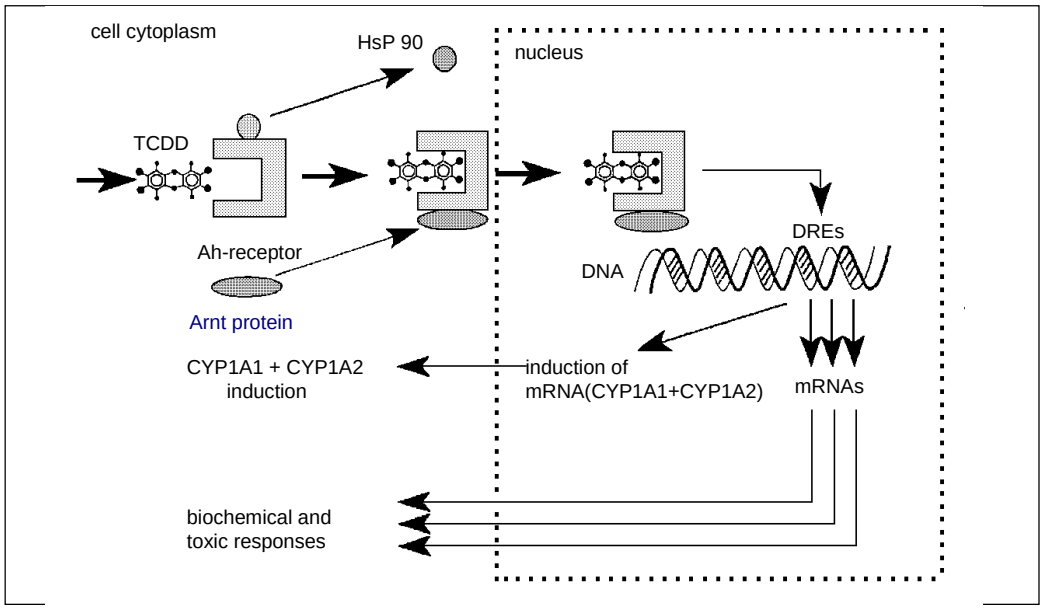

Figure 3 Schematic representation of AH-receptormechanism. See text for explanation. Ah: aromatic hydrocarbon. DRE: dioxin responsive element, HsP: heat shock protein, Arnt: Ah-receptor nuclear translocator, CYP1A1/CYP1A2: cytochrome P450-enzyme or messenger RNA coding for this enzyme.

besides the hydroxymetabolites, namely the methylsulphonmetabolites, have also been observed (Bra94). It is unclear whether these compounds have a dioxin-like effect. The Committee will not be devoting any further attention to these substances either.

2.4 Interspecies differences in sensitivity

From observations made in fish, birds and mammals, it appears that the sensitivity to a 2,3,7,8-substituted PCDD or PCDF can vary widely from species to species. In mammals, the acute toxicity of 2,3,7,8-TCDD between, for example, the guinea pig and the hamster differs by a factor of more than 1000 (Koc85). These differences in species sensitivity depend on the effect being examined. Thus, man and the Rhesus monkey occupy an intermediate position among species as regards sensitivity to acute toxic effects (Gey90). If, however, we look at neurotoxic effects and developmental disturbances, then it appears that primates, including man, are among the most sensitive species (Bow89a, Pet93, Til90).

The extensive research that has been carried out has not, however, produced data that help to explain the substantial differences in species sensitivity (Tuo91). This is true both of the binding of PCDDs, PCDFs and PCBs to the Ah receptor, and of the

effect of the metabolites, because the binding affinity of the metabolites for the Ah receptor is lower than that of the starting substances (Saf92).

binding of the activated receptor complex to the DNA in the cell (Den90, Mas88, Pol82, Saf86, Saf90, Wil90). However, mammalian species with a higher metabolic capacity do appear, in general, to be somewhat less sensitive to the toxicity of these compounds (Ber94a). In addition, the distribution of the substance over the body seems to have a part to play; the explanation for this lies in the size of the fat content, and in the capacity of the liver to induce specific proteins. The body distribution in man differs strikingly from that in much-used laboratory animals such as the rat and the mouse. In rats and mice, an important part of the 2,3,7,8-substituted PCDDs and PCDF is stored in the liver, while in man it is stored in the fat. Monkeys occupy an intermediate position in this regard (Ber94a). The implications of this are not clear at present. On the one hand, it has been determined that, in mammals, an increasing fat content is accompanied by a decreasing sensitivity to acute toxicity (Gey90). On the other hand, storage in the fat reserves leads to an accumulation of the substances in the body in fatty tissue and blood until an equilibrium is reached. Man is therefore at a relative advantage through storing these substances in the fatty tissue, but at a disadvantage as a result of the higher concentrations in the blood caused by the accumulation. Whether harmful effects will ultimately occur is therefore dependent, not just on the degree of exposure, but also on the tissue-specific sensitivity. Regarding species differences in the distribution of PCBs in the body, little is known.

Similarly, species-specific sensitivity plays a part in the assessment of ecotoxicological effects. This has already been partially examined in 2.2, where the Committee pointed out the variations in TEF values between different groups of animal species. For the PCBs in particular, substantial differences in sensitivity between mammals, fish and birds have been established (Bos92, Saf90, Saf94, Wei94).

2.5 Persistence and accumulation

The PCDD and PCDF congeners with chlorine atoms in the 2, 3, 7 and 8 positions, and the highly chlorinated PCBs, are the most persistent in the environment. Dioxins and furans with four chlorine atoms or less are spread through the air, mostly in gaseous form. In the air, the more highly chlorinated PCBs are usually bound to particles, depending on the temperature and, of course, the presence of suitable carrier particles. Gaseous dioxins and furans disappear relatively rapidly through oxidation and decomposition under the effects of sunlight. The half-life of this oxidation process is between ten and fifty days, and that of the photolysis between one and several dozen days. Dioxins bound to particles are, however, either not converted or only slightly converted, and fall back onto the soil, vegetation and the surface water. Dioxins and furans strongly adsorb organic material in the soil and in sediment. In the deeper soil

and the lower sedimentary layers, bacteria and fungi convert dioxins and furans. Higher chlorine substitution leads, in general, to a lower conversion rate. In the soil, the conversion rate varies between one and more than ten years, and in the water between a few days and several hundred days (RIVM93).

Regarding the transport and the fate of the dioxin-like PCBs in the environment, little is known. They are especially associated with soils and sediments, and are thermally and chemically stable. Conversion under the effects of sunlight, followed by slow anaerobic and aerobic biodegradation, appears to be the most significant degradation route for PCBs, for the low-chlorinated congeners at any rate (EPA94a).

Of the PCDDs, PCDFs and PCBs ingested by organisms, only a portion is metabolised. Mammals metabolise PCDDs, PCDFs and PCBs with the help of cytochrome P4501A enzymes through the hydroxylation of carbon atoms. In the case of the persistent PCDD, PCDF and PCB congeners, this process is made more difficult by the fact that the relevant carbon atoms are shielded by the chlorine atoms that are bound to them. This explains why 2,3,7,8-substituted PCDD and PCDF congeners accumulate selectively in tissues with a high fat content and in the liver of higher organisms such as mammals (including man), (fish-eating) birds and fish. The half-life of 2,3,7,8-TCDD in man is approximately seven years (Ber94a). In the case of PCBs also, increasing chlorination is accompanied by a less rapid conversion in the body. This can lead to the accumulation of highly chlorinated PCBs in the food chain (Bar94, Bos95).

Via bioaccumulation, the more persistent PCDDs, PCDFs and PCBs end up in those organisms that stand at the head of the food chain, such as man and predators in aquatic and terrestrial ecosystems. In the opinion of the Committee, both these phenomena — bioaccumulation and persistence — should be taken into account when deriving recommended exposure limits.

Chapter 3

Risk evaluation for man

3.1 Introduction

It has already been indicated in the previous chapter that exposure to dioxin-like substances can, under certain circumstances, harm a person's health. In the following sections, the Committee will subject this possible damage to further scrutiny, a form of analysis known as risk evaluation.

To begin with, the existing knowledge of the adverse effects of exposure to dioxins must be delineated. A key concept in this process is the 'dose', the ingested quantity per unit of body weight (usually expressed in ng or pg per kg). If mixtures of dioxins are involved, then their toxic equivalency (TEQ) is calculated instead, following the procedure described in chapter 2. Tests on rodents and monkeys, and epidemiological studies, have revealed that all sorts of physiological changes and diverse forms of organ damage are associated with exposure of this kind — above a certain dose at any rate. In section 3.2, the Committee summarises the various effects. In as far as data are available on the doses at which the effects are or are not observed, a picture of the relevant dose-effect relationships can be acquired.

In section 3.3, a so-called health-based recommended exposure limit is specified step by step (GR78, GR85b, GR86b). From data on dose-effect relationships, the highest exposure level (dose) at which no adverse effect is perceived is derived; that level is usually indicated by the acronym NOAEL, the No Observed Adverse Effect Level. The NOAEL thus determined is then divided by safety factors, which are used to make allowance for various uncertainties in applying the original data to the

population to be protected. This quotient is the health-based recommended exposure limit. There are, however, agents for which, due to their working mechanism, no NOAEL can be specified. For such agents, a recommended exposure limit is derived on the basis of a risk level laid down by the government (GR81).

Of crucial importance is how the expression 'adverse effect', which forms a part of the NOAEL concept, should be interpreted. In the event of dioxin-like substances, the Committee regards as an 'adverse effect' any observed change that, whether immediately or in the long term, is either harmful to an organism, or alternatively is an adaptation or reaction of the organism that cannot with certitude be regarded as harmless, even if that change remains, for the time being, within the variation of the homeostasis (homeostasis is the capacity of organisms to compensate, within certain limits, for effects that interfere with their functioning). This ambiguity - the fact that we are dealing with changes that may be harmful, or that cannot with certitude be regarded as harmless - forms the starting-point for the derivation of a health-based recommended exposure limit for dioxin-like compounds in section 3.3.1. In section 3.3.2, the Committee compares that value with epidemiological data. Section 3.4 provides information regarding sources of exposure to dioxins, the estimated (daily) dose received by the average Dutch person, groups of persons within the population with a higher estimated exposure, and exposure trends. In section 3.5, the Committee assesses the health-based recommended exposure limit in the light of that exposure data and subsequently makes a number of recommendations.

3.2 Health effects

In this section, we tackle the question: what are the effects that are associated with exposure to dioxin-like compounds? After this, a concise explanation of a series of links that, in the Committee's opinion, represent the nucleus of the 'current state of scientific knowledge' in this field, and that, as a result, must form the basis for the derivation of health-based recommended exposure limits. We are confronted here with very diverse effects that are usually observed in laboratory animals, but that are sometimes also determined epidemiologically. The Committee indicates, where possible, which compounds are brought up for discussion, and what the exposure pattern has been (see also table 3).

3.2.1 Death

Chronic exposure to 12 to 50 ng of TCDD per kg per day in the food of Rhesus monkeys led to death (All77, McN77).

3.2.2 *Metabolic changes*

Enzyme induction

For 2,3,7,8-TCDD, enzyme induction, especially the induction of liver enzymes (CYP1A1, CYP1A2), is the first effect observed as the dose increases . In marmosets that were exposed on a single occasion to 2,3,7,8-TCDD, a clear link was found between the dose administered and the induction of cytochrome P4501A2 activity (Kru90). Also, research using Sprague Dawley rats revealed a relationship between a single oral exposure to 2,3,7,8-TCDD and cytochrome P4501A1 enzyme activity (Kit79). A link of this kind was found between exposure by means of the diet and cytochrome P4501A1/2 activity (Bir95). From the results of the latter study, an NOAEL for the daily dose of 0.3 ng per kg per day can be derived.

The induction of the P450 isoenzymes CYP1A1 and CYP1A2 by exposure to dioxin-like compounds has been demonstrated on many occasions both in the laboratory and in the field; this phenomenon can be used as a biomarker for exposure to these substances. Its physiological significance is, however, unclear. It should be possible for both enzymes to play a part in the steroid-hormone balance (Cha95, Koh93). In mammals, CYP1A1 appears to have a function in the metabolism and in the corresponding detoxification of dioxin-like substances (Ber94a). In pregnant women, CYP1A1 is found in the placenta, and appears to be induced there by dioxin-like substances and polycyclic aromatic hydrocarbons (PAH). CYP1A2 does seem to be a more suitable biomarker for the exposure, however, because this isoenzyme occurs, and is induced in, the human liver.

Thyroid hormone

Exposure of rats in the womb (*in utero*) and via suckling (lactation) to Aroclor 1254, a mixture composed of both dioxin-like and non-dioxin-like PCBs, resulted in a substantial decline in thyroid hormone levels in the plasma and brains of foetuses and the new-born (Bro95). These effects occurred also during exposure to individual non-ortho-PCBs (Mor93). It also appeared, from the data reported in an extensive Dutch infant study, that higher concentrations of PCDDs, PCDFs and PCBs in the maternal milk correlated dose-dependently with lower thyroid hormone contents in the plasma in both mothers and children (Koo94a). In addition, in the children of mothers with higher than average PCDD, PCDF and PCB contents in the maternal milk, slightly increased concentrations of thyroid-stimulating hormone were observed in the plasma compared with the children of mothers with lower than average contents of

PCDD, PCDF and PCB in their milk. All the observed changes in thyroid hormone levels, however, remained within the clinically normal limits. In contrast to the results of this study, an earlier investigation carried out among a smaller group of infants found a slightly increased total thyroxine (TT4) concentration in the blood of children with the higher level of exposure (Plu93b). The reasons for these differences are unclear. It might have something to do with statistical coincidences, given the low absolute differences in TT4 concentrations. It could also be associated with the fact that hormone levels in plasma exhibit a rhythmic increase and decrease, both per day and over longer periods. The fact that dose-dependent changes were observed in the largest infant study suggests that dioxin-like substances are at least partly responsible for the observed effects.

Vitamin K

The presence of PCDDs, PCDFs and dioxin-like PCBs in the maternal milk could result in a vitamin K deficiency and, as a result, an increased risk of haemorrhages in infants (Plu93a). From experimental research (Bou94) it appears that 2,3,7,8-TCDD in predominantly female rats has negative effects on the vitamin K-dependent clotting time of clotting factors in the blood, and on vitamin K concentrations in the liver. The lowest dose of 2,3,7,8-TCDD at which effects on a clotting factor were still observed in the case of female rats was, however, 10 to 35 times higher than the estimated total dose in a human baby that was suckled for eight months. The effects could be caused either directly (via the Ah receptor), or indirectly through hormonal disturbances. Also, non-dioxin-like PCBs have a strong vitamin K-inhibiting effect in laboratory animals.

Vitamin A

Reductions in liver retinoid content (retinoids are specific conversion products of vitamin A) were observed in Sprague Dawley rats that were exposed via their diet to 14 ng of TCDD per kg of body weight per day over a period of 13 weeks (Bir94a). This dose can be interpreted as a LOAEL. The fall in retinoid content is probably due to a change in the metabolism of the retinoids. In the plasma, increases of retinol concentrations are found (retinol is vitamin A in alcohol form). Exposure of rats to dioxin-like PCBs can, in contrast, result in reductions in plasma retinol contents via a different mechanism, namely disturbance of the plasma transport of retinol by PCB metabolites (Ahl92a, Bro91).

3.2.3 Skin toxicity

As is evident from epidemiological studies, there is a consistent link between chloracne and (high) exposure to the compounds trichlorophenol and polychlorinated phenoxyacetic acid contaminated with 2,3,7,8-TCDD. Chloracne is considered as a systemic effect (i.e. one that is distributed over the entire body) of acute and chronic (high) exposure. In an investigation into the details of the industrial accident in Seveso, no dose-effect relationship could be established (Moc91). The doses received by people from the highest exposure group who developed chloracne, especially children, varied from 828 to 56,000 ng per kg of body weight. Those of people from the highest exposure group without chloracne, especially adults, varied from 1770 to 10,400 ng per kg. Also, following exposure to PCDFs, PCBs and polychlorinated quarter phenyls (PCQs) in contaminated cooking oil (the so-called Yusho and Yu-cheng incidents), clinical symptoms arose, such as acne, abnormal skin pigmentation, hypersecretion of sebum by the sebaceous glands, and disturbances in dental formation such as premature tooth eruption. The exposure to 2,3,7,8-substituted PCDFs was estimated at 100-200 ng per of body weight.

In a retrospective study of the consequences of occupational exposure to PCDDs, a correlation was found between the extent of, and the age at the moment of, the exposure and the occurrence of chloracne (Bon89). Exposure to 2,3,7,8-TCDD during foetal development caused effects on ectodermal tissues, such as skin, nails and teeth.

In experimental research, effects of 2,3,7,8-TCDD exposure on skin and teeth were found in adult monkeys, in mice exposed during lactation, and in cell tests on human keratinocytes (special epidermis cells) (Pel92, Pet93). Also, hair loss occurred in monkeys (Sch78).

Only high levels of exposure to dioxin-like substances result in chloracne; chloracne can thus act as a biomarker for disturbances of this type, but is not suitable as a sensitive toxicological end point for the risk estimation.

3.2.4 Reproductive toxicity

By reproductive toxicity (or reprotoxicity) is understood the occurrence of harmful effects of agents upon the reproductive system and the fertility of men and women, or upon the development of their offspring. As the starting-point for determining the reprotoxic effects of PCDDs and PCDFs, the three-generation reproduction study of Murray and colleagues is used (Mur79). From this experiment, an NOAEL can be derived of 1 ng of 2,3,7,8-TCDD per kg of body weight per day (RIVM93).

Male sex organs and sperm

Experimental exposure of male rats and hamsters to 2,3,7,8-TCDD *in utero* and via lactation resulted in a reduction in the daily sperm production, in the numbers of epididymal and ejaculated sperm cells, and in various effects on the male sex organs, such as late descent of the testes and a low weight of the dorsolateral prostate (Bro95). Reductions in the numbers of epididymal sperm cells had already been observed during perinatal exposure to 64 ng of 2,3,7,8-TCDD per kg of body weight (Mab92a, Mab92b). Exposure of male offspring to 2,3,7,8-TCDD *in utero* and via lactation can cause partial feminisation and emasculation. However, the precise effect differs per animal species and per animal strain (Bro95). Exposure of rats to a single dose of 2,3,7,8-TCDD of 12.5 - 50 μg/kg reduced the volume of Leydig cells in the testes dose-dependently (Joh94); this probably explains the changes in the sex hormone balance. Dioxin-related effects on the hypothalamus-hypophysis-Leydig cell system, and on the testosterone synthesis, have been described in publications on experimental research (Pet93).

Similarly, in man, trends in changes in male sex hormone concentrations in the blood appear to be associated with exposure to dioxins (Ege94). In 11- to 14-year-old children who were exposed *in utero* to high concentrations of PCBs and PCDFs during the Yu-cheng incident, a delayed development of the male sex organs has been described (Guo93).

In the wives of Vietnam veterans who had been exposed to high doses of the defoliant Agent Orange (in which 2,3,7,8-TCDD occurred as contaminant), an increased chance of miscarriage, related to the husband’s dose, has been reported (Ste88).

Female sex organs and egg-cells

Female rats and hamsters that were exposed *in utero* and via lactation to 2,3,7,8-TCDD displayed urogenital abnormalities (Bro95, Pet93). In general, exposure to 2,3,7,8-TCDD has anti-oestrogenic effects. High doses of 2,3,7,8-TCDD cause dose-dependent toxic effects in human choriocarcinoma cells, together with a reduced production of oestradiol and progesterone: this is an indication that the functioning of the placenta may be disturbed (Han93).

Adult female Rhesus monkeys that were exposed every day for four years to 5 and 25 ppt (parts per trillion) of 2,3,7,8-TCDD in their food developed endometriosis during the subsequent ten years (Rie93); 5 and 25 ppt agree approximately with doses of, respectively, 0.13 and 0.64 ng per kg of body weight per day. The incidence and seriousness of the endometriosis depended on the dose.

During the accident in Seveso, women received a higher dose of 2,3,7,8-TCDD than the doses administered in the laboratory animal test just referred to (Boi94, Bow89a). Regarding the incidence of endometriosis among them, no epidemiological data have become available to date. However, a decline in the prevalence of breast and endometrial cancer has been observed (Ber93), possibly on account of the anti-oestrogenic effect of 2,3,7,8-TCDD (DEN94); from this data, however, it is impossible to deduce a dose-effect relationship (Ber93).

The various reprotoxic effects following perinatal exposure to 2,3,7,8-TCDD can, it seems, be caused in two ways: directly via the Ah receptor mechanism, or indirectly via changes in sex hormones such as oestrogens, androgens and other hormones. The effects of PCDDs, PCDFs and dioxin-like PCBs on the sex organs, reproduction and reproductive behaviour is probably caused by an oestrogenic or anti-oestrogenic effect, or indirectly by changes in the balance of oestrogens, testosterone or the thyroid hormone.

In a number of recently published articles, attention is drawn to the effects on the development and reproduction of animals and man of hormone-disturbing substances in the environment (Bir94b, Col93, Dav93, Dod93, McK94, Sto94). It is possible that the anti-oestrogenic effect of 2,3,7,8-TCDD and the oestrogenic activity of some PCBs and PCB metabolites could play a part in this. Also, exposure to 2,3,7,8-TCDD and PCBs could indirectly affect sperm production and the size of the testes via changes in the thyroid hormone balance (Sto95).

3.2.5 Developmental toxicity

Data on laboratory animals

In experimental research, changes in cognitive and sexual behaviour and in the motor function were observed in rats, mice and monkeys following perinatal exposure to 2,3,7,8-TCDD or PCBs (Bow89b, Bro95, Til90). Cognitive changes and motor effects are, in general, long-term, and remain present during adulthood. It is possible that they are caused by changes in neurotransmitter levels in the central nervous system. The cognitive capacity of Rhesus monkeys that were chronically exposed to 2,3,7,8-TCDD in their food, and, indeed, to a dose of 0.13 and 0.64 ng per kg per day (that is, respectively, 0.3 and 1.5 ng per kg of body weight per day for the baby), appeared to be affected. These effects involved impaired learning behaviour and disturbances in social interaction between mothers and offspring (Bow89b). Exposure of rats *in utero* and via lactation to the PCB mixture Aroclor 1254 resulted in changes in synaptophysine and calcineurine, biomarkers for structural and functional brain development. Also, after exposure to this substance, significant falls in thyroid

hormone levels were observed in the foetal and neonatal plasma, and in the animals' brains (Bro95); the same thing occurred after exposure to individual non-ortho- and mono-ortho-PCBs (Mor93).

Exposure of pregnant monkeys, guinea pigs, rabbits, rats, hamsters and mice to 2,3,7,8-TCDD (Pet93) or dioxin-like PCBs (Til90) can lead, it seems, to prenatal death. This effect depends on the dose and the moment of exposure. In addition, the sensitivity appears to decrease from monkey and guinea pig to rabbit, rat and hamster, with the mouse as the least sensitive species. In general, the prenatal death induced by 2,3,7,8-TCDD is associated with toxic symptoms in the mother, expressing itself in, among other things, a reduced maternal weight increase. In monkeys, however, exposure to 2,3,7,8-TCDD can cause prenatal death without symptoms of poisoning arising in the mother (Pet93). This appeared, for example, from research on Rhesus monkeys that were exposed for a period of four years to 0.64 ng of 2,3,7,8-TCDD per kg per day in their food (Bow89a, Hon89); in pregnant Rhesus monkeys exposed on several occasions to a dose of 22 or 111 ng per kg, prenatal death occurred only in the higher-dosage group (McN84). In an experiment on rats, no prenatal deaths occurred after exposure of the mothers to 30 ng of 2,3,7,8-TCDD per kg per day between day 6 and day 15 of the pregnancy, while higher doses caused an increase in prenatal death, resorptions, oedema and gastric haemorrhages (Spa71).

Epidemiological data

Following a high exposure of infants to PCBs, PCDFs and PCDQs via the mothers, as occurred in the Yusho and Yu-cheng incidents in, respectively, Japan and Taiwan, diverse effects were observed: a low birth weight, hyperpigmentation, an increased incidence of bronchitis, and a long-term retardation in development (Rog88). These effects arose, above all, in the first and second children.

More recent Dutch research reveals that the most important effects on the development of infants following a relatively low exposure to PCBs, PCDDs and PCDFs *in utero* and via lactation are - sometimes temporary - fluctuations in thyroid hormone levels (in the blood) and in neurological, psychomotor and immunological scores (Hui95a, Hui95b, Jac85, Koo94a, Koo95a, Plu93a, Rog87, Wei95). In the largest Dutch study, the research population consisted of 200 breast-fed children and their mothers, and 200 formula-fed children and their mothers. One half of each group came from the Rotterdam region, the other half from the Groningen region. The average exposure of the breast-fed children amounted to 0.265 ng TEQ_{TOTAL} per kg of body weight per day. In interpreting the research data, a correction has been made to allow for various disruptive variables, including those associated with the socio-economic status of the mother.

The neurological development of the infants (measured in the Prechtl/Touwen test) had a similar course in both groups both 2 weeks and 18 months after birth. The neurological optimality score 2 weeks after birth was lower in the group that was exposed to larger quantities of PCBs, PCDDs and PCDFs in the maternal milk. At the age of 18 months, only the prenatal exposure to PCBs had a negative relationship with the neurological condition (Hui95a, Hui95b).

In a visual recognition test (the Fagan Infantest), no effects were observed in respect of perinatal exposure to PCBs, PCDDs and PCDFs. At the age of 7 months, visual recognition showed a positive correlation with the duration of the breast-feeding. This positive influence can probably be attributed to natural hormones and other hydrophobic ingredients in the maternal milk (Koo95a).

In a subgroup, the psychomotor and mental development were measured at different ages (in the Bayley scale). At the age of 3 months, a higher prenatal exposure to PCBs correlated with a lower psychomotor score; at the age of 7 months, breast-feeding infants scored higher on the psychomotor scale than the formula-fed infants. However, the score of the 66% most highly exposed breast-fed children (those who had undergone an exposure of more than 0.8 ng TEQ_{TOTAL}) was similar to that of the formula-fed children; from this finding it can be deduced that the postnatal exposure had a negative effect. In children aged 18 months, neither the mental nor the psychomotor score was related to perinatal PCB, PCDD and PCDF levels, or to the duration of the breast-feeding. At this age, socio-economic factors have a decisive influence on the score (Koo95b).

No significant relationship was observed between the perinatal exposure of children to PCBs, PCDDs and PCDFs and the occurrence of infections of the highest and lowest bronchial tubes (rhinitis, bronchitis, tonsillitis and otitis) or of humoral immunity (antibodies against mumps, measles and German measles). Counts and biochemical analysis of white blood cells (monocytes, granulocytes and lymphocytes), however, showed that there is a relationship with exposure. A higher prenatal exposure was associated with an increase in specific T lymphocytes both at birth and at the age of 18 months. A higher prenatal exposure was accompanied by fewer monocytes and granulocytes (measured at the age of 3 months; Wei95).

In the case of the infants, the link between the current exposure and the content of thyroid hormones in the plasma has also been examined. As the Committee noted in section 3.2.2, a higher burden of dioxins and PCBs correlated with lower plasma levels of TT3 (triiodothyronine) and TT4 (total thyroxine) and with higher levels of TSH (thyroid-stimulating hormone). In the mothers, during the measurement period (2 months), the plasma levels of TT3 and TT4 were also reduced (Koo94b, Sau94).

3.2.6 *Immunotoxicity*

By immunotoxicity is understood the occurrence of harmful effects on the system of natural defences (immune system) in man or animal as a result of exposure to agents. That effect can increase sensitivity to infections. Also, exposure to immunotoxic substances can increase the chances of certain types of cancer occurring, and favour the occurrence of allergies and autoimmune diseases (GR91b).

The effects of 2,3,7,8-TCDD on the immune system vary per animal species and laboratory animal strains. Direct immunotoxic effects of 2,3,7,8-TCDD are atrophy of the thymus and the spleen, and of the peripheral lymph nodes. The seriousness of the consequences is dependent on the age because the functioning of the thymus decreases with age.

Chronic exposure of female Rhesus monkeys to 0.642 ng 2,3,7,8-TCDD per kg per day in their food brought about changes in T-cell subpopulations; these did not however lead to an increased incidence of mortality, infections or cancer. In the offspring of this exposure group, an increased antigenic response was detected that correlated with 2,3,7,8-TCDD contents in tissues (Hon89). In marmosets, an increase in peripheral lymphocyte subpopulations was demonstrated after exposure to 2,3,7,8-TCDD in doses of 0.3 ng per kg per week for 24 weeks, followed by 1.5 ng per kg per week for 6 weeks. This exposure agrees with a chronic 2,3,7,8-TCDD intake of 0.14 ng per kg per day. After single doses, a decline in the lymphocyte populations was predominantly found (Neu92).

In mice and rats, it was only at the higher doses, from 100 to 3000 ng 2,3,7,8-TCDD per kg, that effects on the immune system were found. At doses of 10 to 300 ng per kg per week, no immunosuppression arose; in mice receiving a single dose of 10000 ng per day, however, these effects did occur (Han94).

In occupationally exposed men and other populations with an increased 2,3,7,8-TCDD exposure, no changes in peripheral white blood cell populations were found (Neu92, Neu93). In humans who were accidentally exposed to relatively high doses of 2,3,7,8-TCDD, as in Seveso and Missouri, no or conflicting effects on the immune system were observed (Hee95).

3.2.7 *Neurotoxicity*

Dioxin-like PCBs cause adverse effects on neurochemical parameters in rats after exposure *in utero* and via lactation: these effects include changes in biogenic amines and in dopaminergic neurotransmitter levels (Bro95, Til90). These effects did not arise

following exposure of adult rats and non-anthropoids (See94).* It has already been pointed out in section 3.2.5 that exposure to Aroclor 1254 can affect structural and functional brain development (Bro95).

3.2.8 Carcinogenicity and mutagenicity

In vivo and *in vitro* research involving laboratory animals and cells has not provided any indication of a direct interaction of dioxin-like compounds with DNA (RIVM93). Epidemiological research seems to give conflicting results. In the case of employees who were exposed to 2,4,5-trichlorophenoxyethanol contaminated with 2,3,7,8-TCDD, an increase in chromosomal abnormalities in peripheral lymphocytes was noted. Such abnormalities were not found in persons who were exposed to herbicides contaminated with 2,3,7,8-TCDD in Vietnam and Seveso (RIVM93). According to so-called initiation/promotion research, 2,3,7,8-TCDD has strong promoter properties in the liver of rats; an initiating effect has not been demonstrated (RIVM93).

Laboratory animal research has revealed that orally administered 2,3,7,8-TCDD produces liver and thyroid gland tumours in mice and rats. In rats, carcinomas in the nasal and oral cavity and in the lungs were also observed. The Committee on Carcinogenic Risk Assessment, whose opinion on the Criteria Document on Dioxins has been included in this report as Annex A, thinks that the research of Kociba and colleagues (Koc78) is a suitable basis for the risk estimation concerning the carcinogenicity of 2,3,7,8-TCDD (see Annex A).

3.3 Health-based recommended exposure limit

The data given in the previous section constitute the empirical source on which we must draw in order to derive a health-based recommended exposure limit for a chronic exposure to dioxin-like compounds. As was noted in the introduction to this chapter, to do this we need to know the NOAELs for the various adverse effects reported. Many of the laboratory animal experiments described can be used to determine such NOAELs or, where that appeared to be impossible, so-called LOAELs (Lowest Observed Adverse Effect Levels). Table 3 provides an overview of the relevant studies (a selection of the data discussed in 3.2). In section 3.3.1, the Committee derives, from the results of these studies, the health-based recommended exposure limit that it is seeking. In section 3.3.2, the Committee offers an interpretation of the value arrived at in the light of the epidemiological information also furnished in 3.2.

* The non-dioxin-like di-ortho-PCBs also produce changes in neurotransmitter levels after exposure of rats *in utero* and via lactation, as well as in adult rats and monkeys (See92).

Table 3 NOAELs and LOAELs ('no-' and 'lowest-observed-adverse-effect levels') of 2,3,7,8-TCDD for a number of mammalian species in different experimental design with doses between 1 and 100 ng/kg per day. m: mother animal. See also EPA94b.

species	experimental design	NOAEL	LOAEL	effect	reference
Rhesus monkey	0.5 ppb in food, 9 months		12 ng/kg/d	death	All77
Rhesus monkey	2 ppb in food, 61 days		50 ng/kg/d	death	McN77
Rhesus monkey	0.05 ppb in food, 20 months		1.5 ng/kg/d	hair loss	Sch78
Rhesus monkey	9x22 ng/kg m, 20-40th day of pregnancy	9x22 ng/kg m		prenatal death	McN84
Rhesus monkey	5 and 25 ppt in food m, 4 years		0.126 ng/kg/d	object recognition, juvenile	Bow89b
Rhesus monkey	5 and 25 ppt in food m, 4 years		0.642 ng/kg/d	prenatal death	Bow89a, Hon89
Rhesus monkey	9 x 111 ng/kg m, 20-40th day of pregnancy		9x111 ng/kg m	prenatal death	McN84
Rhesus monkey	25 ppt in food m, 4 years		0.642 ng/kg/d m	change in lymphocytes	Hon89
Rhesus monkey	5 ppt in food m, 4 years		0.126 ng/kg/d m	endometriosis	Rie93
Marmoset monkey	3 ng/kg, 1 x orally		3 ng/kg	induction of CYPIA2	Kru90
Marmoset monkey	0.3 +1.5 ng/kg/wk x 24 + 6 weeks		0.135 ng/kg/d chron.[a]	change in lymphocytes	Neu92
Spr. Dawley rat	2 ng/kg, 1 x orally	0.6 ng/kg	2 ng/kg	induction of CYPIA1	Kit79
Spr. Dawley rat	1,10 ng/kg/d, orally, 2 yrs	1 ng/kg/d	10 ng/kg/d	porphyria	Koc78
Spr. Dawley rat	14-1024 ng/kg/d, orally, 3 months	< 14 ng/kg/d	14 ng/kg/d	less vit. A	Bir95
Spr. Dawley rat	14-1024 ng/kg/d, orally, 3 months	0.3 ng/kg/d[a]	14 ng/kg/d	induction of CYPIA1	Bir95
Spr. Dawley rat	14-1024 ng/kg/d, orally, 3 months	0.5 ng/kg/d[a]	14 ng/kg/d	induction of CYPIA2	Bir95
Spr. Dawley rat	27 ng/kg m, chronic	1 ng/kg/d		prenatal death	Mur79
Spr. Dawley rat	30 ng/kg/d m, 6-15th day of pregnancy	30 ng/kg/d m		prenatal death	Spa71
Holtzmann rat	64 ng/kg/d m, 15th day of pregnancy		64ng/kg/d m	decrease in male reproductive capacity	Mab92a,b
C57B1-mouse	1 ng/k/wk, 4 wk i.p.		1 ng/kg/wk	immunosuppr., low CTL generation	Cla83
B6C3F1-mouse	10 ng/kg, 7 days after fertilisation		10 ng/kg, d 7	incr. viral infec.	Leb94
guinea pig	1 ng/kg/d, 8 wk		1 ng/kg/d	immunosuppression, lower response tetanus toxin	Vos73
Spr. Dawley rat	100-10000 ng/kg/d, 30 d		100 ng/kg/d	lower serum glucose	Zin73
Spr. Dawley rat	calculated with data from Tri92 and Sew93; 3.5-10,7-35,7-125 ng/kg/d, 30 wk	0.01 ng/kg/d	0.1 ng/kg/d	CYP1A1 induction	Koh93
		0.1	1	CYP1A2 induction	
		0.1	1	Ah receptor induc.	
		0.1	1	EGF recept. induc.	
		0.1	1	oestrogen recept. induction	

[a] calculated

3.3.1 *Health-based recommended exposure limit on the basis of laboratory animal data*

The initial health-based recommended exposure limit for the intake of dioxin and dioxin-like compounds in the Netherlands, namely 4 pg per kg per day, was based on an LOAEL value drawn from the research of Kociba and colleagues (Koc78); the LOAEL of 1 ng per kg per day arrived at was divided by the usual safety factor of 10 to allow for the inter- and intraspecies variation, and by an extra factor of 2.5 because it was an LOAEL instead of an NOAEL. Later on, WHO recommended 10 pg per kg per day as a recommended exposure limit (Ahl92b, WHO91). This value rested on an NOAEL for man, derived on the basis of pharmacokinetic considerations, of 100 pg per kg per day; in the absence of data relating to reproductive toxicity and variation in sensitivity between human beings, 10 was selected as the safety factor (The91b).

Since the publication of the study by Kociba (Koc78), other effects than cancer have been examined. The data in table 3 show that, for some of these effects - using the term ‘adverse effect’ as it is understood by the Committee (see 3.1) - NOAELs or LOAELs have been found that are lower than 1 ng per kg per day. The induction of the enzymes CYP1A1 and CYP1A2, and a more obviously adverse effect such as the increase of endometriosis, serve to illustrate that.

Regarding the experiments mentioned in table 3, the Committee considers that the experiments reported in Bow89a, Bow89b, Neu92 and Rie93 are the most relevant basis for the derivation of a health-based recommended exposure limit. The Committee has two reasons for holding this view: the laboratory animals were primates (and therefore are relatively close to man) and the observed effects are, in the Committee’s eyes, adverse. The Committee is aware that this view is not adhered by everyone (of the scientific community), but it wishes to point out that, in an evaluation carried out by the Environmental Protection Agency in the United States, importance is also attached to these research data (EPA94b).

Using the animal tests just referred to as a basis, an LOAEL can be determined. In the one study, a change in cognitive development in baby Rhesus monkeys was noted; the effect arose in the case of a 2,3,7,8-TCDD dose of 0.1 ng per kg per day in the mothers (which themselves had endometriosis; Bow89a, Bow89b, Rie93, Sch89). The other study showed a change in lymphocytes in marmosets at a dose of 0.13 ng per kg per day (Hon89, Neu92).

How do we make the transition from an LOAEL to an NOAEL, the quantity from which the recommended exposure limit must be derived? The best way of accomplishing this, in the opinion of the Committee, is to study the course of the dose-effect relationships for all kinds of effects within the dosage range lying between

about 0.1 and 10 ng per kg per day. Many experiments point out that the gradient of the curves in that dosage range is quite small (EPA94b). On the basis of models of the dose-effect curves for the different effects that have been observed in rats in the low dosage area, the Committee thinks that the relevant NOAELs for laboratory animals work out - at the most - at a factor of 2 lower than the corresponding LOAELs. The LOAEL of 0.1 ng per kg per day for primates referred to above therefore corresponds to an NOAEL of 0.05 g ng per kg per day (50 pg per kg per day).

In selecting safety factors, the Committee has allowed itself to be guided by the following considerations. Using the data relating to rats as a starting-point, 10 is the usual safety factor for the interspecies variation (the possible difference in sensitivity between species). Pharmacokinetic characteristics of PCDDs and PCDFs indicate, however, that, in certain respects (e.g. the way in which these substances are distributed between the liver and the fatty tissue), the monkey occupies a position somewhere between the rat and man (Ber94a; see also section 2.4). For these reasons, the Committee is proposing, in this case, a safety factor of 5. The intraspecies variation (the possible difference in sensitivity within a species and, in this case therefore, between humans) is, according to the Committee, adequately taken into account when the standard safety factor of 10 is used.

Accordingly, the Committee derives as a health-based recommended exposure limit for man 0.1 ng of 2,3,7,8-TCDD per kg per day (the LOAEL from experiments with monkeys), divided by 2 (to make the transition from LOAEL to NOAEL), divided by 5 (taking into consideration the interspecies variation), and divided by 10 (taking into consideration the intraspecies variation), giving a result of 0.001 ng per kg per day (or 1 picogram of 2,3,7,8-TCDD per kg per day). Given the similarity of their effects, the Committee considers this recommended exposure limit to be applicable also to the intake of mixtures of diverse dioxin-like compounds expressed in the TEQ_{TOTAL}.

3.3.2 Health-based recommended exposure limit in the light of epidemiological data

Table 4 provides an overview of the results of the — in the opinion of the Committee — most important epidemiological investigations into the consequences of exposure to PCDDs, PCDFs and dioxin-like PCBs. The Committee attaches much importance to the results of the research conducted among infants in Rotterdam and Groningen, which is described in section 3.2.5 (Hui95a, Hui95b, Koo94a, Koo95a, Plu93a, Plu93b, Wei95), first because the study deals with the consequences of long-term exposure to low doses - the situation to which the health-based recommended exposure limit relates - and, second, on account of the (as is generally assumed) increased sensitivity of infants (and foetuses) to harmful substances. These effects are associated with the building up and development of organ systems, and - as was noted in the

infant study - are among the first that become apparent: this makes them a sensitive measuring instrument.

With reference to section 3.2.5, the Committee here recapitulates the most important results of the research referred to in that section. The first point to make is that no link has been found between serious, clinically relevant abnormalities in the development of new-born children and their degree of exposure to PCDs, PCDFs and PCBs. However, the infants with the highest prenatal exposure over a period of some time did have a lower neurological optimality score, a lower psychomotor score, and an altered immune function parameter; among the infants with the highest postnatal exposure, subtle signs of a suboptimum neurological development were observable, as well as a slight retardation in psychomotor development, changes in the thyroid hormone status, and changes in immunological functions. Although all the differences referred to fall within the range of the clinical reference values, the Committee thinks that their harmlessness to health in the longer term has not been established. In conformity with the definition of 'adverse effect' given in section 3.1, such effects are, in the Committee's opinion, undesirable.

Table 4 shows that the median daily dose expressed in the form of TEQ_{TOTAL} in the case of the mothers of the infants suffering the highest exposure levels amounts to 0.003 ng per kg per day. The fact that, at this dose, effects still arise in infants offers, in the opinion of the Committee, further support for the health-based recommended exposure limit of 0.001 ng per kg per day. In the mothers themselves, physiological effects related to the exposure to dioxin-like PCBs were also observed, i.e. changes in thyroid hormone levels. The significance of this for health is, in the Committee's opinion, still unclear.

3.4 Exposure

3.4.1 General population

Small quantities of dioxins can arise during the combustion of organic material; these compounds have therefore probably always been present in the environment. However, modern industrial activities and combustion practices have caused the concentrations to increase substantially (EPA94a).

For man, the consumption of animal fats is the most important source of exposure to PCDDs, PCDFs and PCBs (RIVM91a, RIVM93). Here we are thinking especially of fats in milk and cheese, of fish oil in industrially manufactured foodstuffs, and of other animal fats in meat products.

According to the study by Theelen (The89, The91a), the estimated median daily dose of PCDDs and PCDFs in the Netherlands, expressed as $TEQ_{DIOXINS}$, is 0.002 ng

Table 4 Body burden in ng TEQ per kg of body weight and effects in man (see also EPA94b).

circumstances of the exposure	body burden	effect	reference
Yusho en Yu-Cheng incidents	45-3000 ng/kg	chloracne	Rya90, Bec89
Yu-Cheng incident	1460 ng/kg[a]	lower birth weight less growth retarded development lower EGFR activity in placenta higher CYP1A1 activity in placenta	Luc91 Guo94 Rog88 Luc91 Luc91
Seveso incident	109-7000 ng/kg[b]	increase in cancer	Ber93, Fin91
Chemical industry	83 ng/kg[c]	lower testosterone concentration	Ege94
US Air Force Vietnam	14 ng/kg	decrease in size of testes	Roe91
Background exposure of Dutch mothers: $3x10^{-3}$ ng/kg/d TEQ_{TOTAL} (P50) Background exposure of Dutch infants: 265 x 10^{-3} ng/kg/d TEQ_{TOTAL} (average)		suboptimal neurological development retarded psychomotor development change in immune system change in thyroid metabolism	Hui95a, Hui95b Koo95a Wei95, Koo94b, Sau94
Background exposure of Dutch infants: 257 x 10^{-3} ng/kg/d TEQ_{TOTAL} (average)		increased total thyroxine	Plu93a

[a] Body burden based on quantity of 2,3,4,7,8-pentachlorine-dibenzofuran (TEF=0,1) and 1,2,3,4,7,8-hexachlorine-dibenzofuran in the placenta. The basic assumption here is a fat content of the placenta of 1% (Bec94). 21% of the body weight of females is accounted for by fat.

[b] Calculations based on TCDD quantities in serum, taking a first-order elimination kinetics and a half-life of 7.1 years as starting-points. Body weight: 70 kg, 22% fat.

[c] Extrapolation by Ege94 on the basis of a half-life of 7.1 years and a background level of 60 ng/kg TEQ. The average employee weighs 70 kg, of which 15% is body fat.

per kg of body weight per day (the TEQ is calculated using the $TEF_{DIOXINS}$ values from table 1). Of this quantity, 98% is absorbed via the diet and the other 2% via inhalation, through the skin, and by ingestion of contaminated air-borne and soil particles. The study by Liem, which is based on the National Food Consumption Survey of 1988, also offers exposure data (RIVM91a). According to this study, the median daily dose for persons aged 20 to 70, expressed as $TEQ_{DIOXINS}$, is 0.001 ng per kg per day; for persons under 20, this figure is higher.

The daily dose of (non-ortho-)PCBs in adults, expressed as TEQ***$_{PCB}$*, is 0.001 ng per kg per day. For these compounds also, the daily dose in the 0-20 age group is higher.

* The authors of RIVM91 ascribe the following TEF***$_{PCB}$ values to the non-ortho-PCBs: 3,3',4,4'-TCB (77), 0.01; 3,3',4,4',5-PentaCB (126), 0.1; 3,3',4,4',5,5'-HexaCB (169), 0.005.

If the toxic equivalent values of all the dioxin-like compounds are added up to form a TEQ_{TOTAL}*, then the median TEQ_{TOTAL} dose for the population aged 20 and over is 0.002 ng per kg per day; the 95th percentile is 0.004 ng per kg per day. According to the same study, in 1% of the children aged between 1 and 6, the 'TEQ_{TOTAL} dose' amounts to more than 0.01 ng per kg per day (RIVM91a). Other groups of persons with a higher than average exposure are those involved in the production or processing of phenoxyacetic acid herbicides, those living in the vicinity of a point source who consume locally cultivated food, and recreational fishermen who consume large quantities of self-caught fish, especially freshwater fish.

3.4.2 *Infants*

As has already been mentioned above, some groups of the population receive a relatively large daily dose. This is true in particular of breast-fed infants. Their exposure is almost entirely determined by the contents of dioxin-like substances in the mother. In recent publications, Dutch researchers consider the PCB concentrations measured in the mothers' peripheral blood and in the blood of the umbilical cord to be a measure of the degree of exposure of the infants before birth (Hui95a, Hui95b, Koo94a, Koo95b, Wei95). For their exposure after birth, the PCDD, PCDF and PCB concentrations in the maternal milk and in formula-feeding are considered determining. The contents in formula-feeding, though, appear to be negligible; that is because, in these, the animal fats are replaced by vegetable fats.

Regarding the exposure of mothers, the exposure of infants, and the relationship between the two, the Committee is able to provide the following information. From a dietary questionnaire and reference data relating to $TEQ_{DIOXINS}$ and TEQ^{***}_{PCB} in foodstuffs in the Netherlands (RIVM91a), it was possible to calculate that the median dose via food of mothers during their pregnancy was 0.003 ng per kg per day (Hui95c). The quantity of dioxin-like compounds in the maternal milk is, however, the result of the accumulation of these substances over a period of, on the average, 30 years. The transfer of the relevant compounds from mother to foetus is also determined by this.

The concentrations of dioxin-like substances in the blood of the umbilical cord and in the maternal blood plasma seem, when related to the quantity of fat in the blood, to be the same. The contents of PCDDs and PCDFs in the fat of a child amount at birth to 20% of that of the mother (Wij90). Given an average total fat mass of 13 kg in the mother and 500 g in the child, this means that 0.8% of the mother's body burden is transferred prenatally to the child. The transfer after birth depends upon the relevant concentrations in the maternal milk, and upon the duration of the breast-feeding.

* The TEQ_{TOTAL} values have been calculated here as $TEQ_{DIOXINS}+TEQ^{***}_{PCB}$.

In the Dutch maternal milk study referred to above, the following contents were found (median values in ng per kg of fat): $TEQ_{DIOXINS}$: 30; TEQ-non-ortho-PCBs: 16; TEQ-mono-ortho-PCBs: 15; TEQ-di-ortho-PCBs: 5. These results are more or less the same as the maternal milk contents revealed in an earlier study carried out in the Netherlands (Bro95). The 95th percentile of the combined concentration of PCDDs, PCDFs and dioxin-like PCBs in maternal milk fat, expressed as the toxic equivalent (TEQ_{TOTAL}), amounts to 113 ng per kg. The average value of the TEQ_{TOTAL} is 68 ng per kg of milk fat (Hui95a, Koo94a). In the case of breast-feeding, that corresponds to 130 ml of milk per kg of body weight per day, and a milk fat content of 30 g per litre, that means an average daily dose, expressed as a TEQ_{TOTAL}, of 0.265 ng per kg of body weight per day for the infant. The total quantity taken in by breast-fed infants depends, as has been noted, on the duration of the breast-feeding (in the Netherlands, usually between six weeks and six months). On the average, the mother transfers more than 10% of her body burden of dioxins to the infant via breast-feeding; in the case of PCBs, that is (in the case of a breast-feeding period of three months) approximately 20%. During this period the child takes in 9% of the average lifelong dose of PCBs (Dua94).

From the above it appears that the exposure of infants to PCDDs, PCDFs and PCBs could be restricted by limiting breast-feeding, and administering more formula-feeding. It has, however, already been known for some considerable time that breast-feeding has a positive influence on the development of the infant compared with formula-feeding. This was confirmed in the recent Dutch study: the Committee draws the reader's attention to the findings regarding visual recognition, and mental and psychomotor development (3.2.5). In earlier advisory reports (GR85a, GR86a and GR91a), the then Committee on Maternal Milk of the Health Council carried out an extensive comparison of breast-feeding and artificial feeding. They included in this study the naturalness of breast-feeding, hygiene, anti-infection characteristics, the relation between mother and child, the composition of breast- and formula-feeding, and the weight regulation of the infant. On the basis of this comparison, the Committee on Maternal Milk concluded: 'On scientific grounds, there is, in the Netherlands, no reason to express a clear preference for either of the two ways of feeding. The (expectant) mother should therefore be left to weigh up for herself the question of how she wants to feed her child.' (GR91a). The Committee on Risk Evaluation of Substances/Dioxins sees no cause to revise this conclusion.

3.4.3 Trends in exposure

As has already been said, industrialisation has been accompanied by an increased emission of dioxin-like substances. Analyses of sedimentary layers reveal an increase

in PCDD and PCDF concentrations from the 1920s to the end of the 1970s (Smi92). At present, however, the concentrations in the environment do appear to be decreasing; this is the result of changes in industrial practices, and through improved management of the emissions (Rap92). Examples of these changes and control measures are: a reduced use of halogenated fuel additives, process changes in pulp and paper factories, emission limits for incinerators, and a reduction in the use of chlorinated phenol (semi)-products. Measures for reducing the emission of, among other things, waste incineration plants - responsible in 1991 for 80% of the total emission of PCDDs and PCDFs in the Netherlands (RIVM92c) - will result in a notable reduction in deposition. Locally increased contents, such as in the milk of cows that are grazing in the vicinity of waste incineration plants, will therefore become something of the past. However, due to the persistent character of dioxin-like substances, contamination of the environment by these compounds will decrease only slowly. The Committee does not anticipate, therefore, that the average exposure of the Dutch population via the diet will decline very substantially over the next few years.

Regarding the change in the concentration of the separate PCBs in the environment, no data are available. Thanks to measures to limit the production and use of PCBs, the emission of dioxin-like and non-dioxin-like PCBs into the air, water and soil in the Netherlands was halved in the period between 1980 and 1990 (RIVM95a). PCBs now enter our environment principally via the Great Rivers, and via atmospheric deposition from outside the country. The relative importance of dumping and leakage, and the unintended production of PCBs, has increased.

The commission is unaware of the results of any research into trends in dioxin contents in food in the Netherlands. However, a 10%-30% reduction in the contents of the important PCDDs and PCDFs in maternal milk in the Netherlands between 1988 and 1993 has been established (Lie95). The same trends are observable in Germany and Scandinavia (Nor90, Ald94). The concentrations of non-ortho- and mono-ortho-PCBs in the maternal milk, which contribute approximately 50% to the exposure, have remained stable over the last 15 years. In Sweden, the concentrations of non-ortho-PCBs in maternal milk fell between 1972 and 1980; after that, until 1989, they stabilised. The contents of non-dioxin-like PCBs in maternal milk did not decrease in the period 1988 to 1993 either in the Netherlands or in Sweden (Lie95).

3.5 Conclusions and recommendations

The Committee concludes that, on the basis of laboratory animal data, a health-based recommended exposure limit for man of 1 picogram TEQ_{TOTAL} per kg of body weight per day can be derived. Set alongside the data relating to exposure in the Netherlands, this means that, in the case of what is now being found to be background exposure - in

other words, exposure that cannot be directly influenced - adverse effects on health cannot be ruled out.

In the opinion of the Committee, reliable data regarding the consequences of pre- and postnatal exposure to dioxin-like compounds (see table 4) support this conclusion, or, at least, do not contradict it. The Committee would, nonetheless, like to point to a number of deficiencies and shortcomings. We have, for example, no epidemiological information regarding groups of persons without any exposure to PCDDs, PCDFs and PCBs. Also, the data relating to exposure and effects are sometimes deficient. The Committee considers it important to achieve a greater degree of clarity regarding these matters, especially in the case of infants. It therefore recommends that continued monitoring of the infants from the Rotterdam and Groningen regions: this measure would bring us nearer to determining the precise significance for health of the changes, discussed above, that occur during the first stage of life.

In the opinion of the Committee, the exposure of children before birth and during the breast-feeding period can best be restricted by employing an exposure norm for the mother and, thereby, for the whole population. Direct limitation of the exposure of the infant via a reduction of breast-feeding is, in the Committee's opinion, not the right way. It has already been known for some considerable time - and this is something that comes up again in the recent study - that breast-feeding offers young children some advantages compared with the alternative of formula-feeding. The Committee points to the results of tests for visual recognition, and mental and psychomotor development. In the opinion of the Committee, there is no reason to restrict the freedom of parents in making a choice between breast- and formula-feeding for their child.

Chapter 4

Risk evaluation for ecosystems and farm animals

4.1 Introduction

It is not only man that can suffer damage through exposure to dioxin-like compounds, but ecosystems as well. Once again, estimation of the possible damage and the derivation of recommended exposure limits with a view to risk management is done in stages. For this risk evaluation, data on the toxicity of, and the actual exposure to, the different substances are required. In undertaking an evaluation of this kind, diverse environmental compartments and organisms must be taken into consideration. In particular, the Committee makes a distinction between aquatic and terrestrial ecosystems. Aquatic organisms, including fish, and fish-eating birds and mammals form part of the first of these ecosystems. The compartments via which they can be exposed are 'water' and 'sediment'. The latter compartment relates to soil organisms, such as the earthworm, and birds and mammals that forage on this type of organism.

In section 4.2, the Committee investigates the balance of distribution between the various environmental compartments involved, the accumulation processes in organisms that live in these compartments (their 'internal exposure'), and the bioaccumulation in predators that are exposed via the food chain. Toxicity data for the different organisms are considered in section 4.3.

This inventory provides the basis for deriving the ecotoxicological recommended exposure limits. The starting-point is provided by those empirically established exposure levels at which no effect on population level that could be characterised as adverse is observed — the NOEC values (No Observed Effect Concentration). Each of

Table 5 Calculation methods for the derivation of ecotoxicological recommended exposure limits for the aquatic system (omitting uncertainty factors).

	water	sediment	used by
aquatic organisms	$NOEC_{water}$	internal $NOEC/BSAF_{fish}$	Committee
		$NOEC_{water} \times K_{oc}$ (or K_{ow}) $\times f_{oc}$	RIVM
birds and mammals	$NOEL_{predator} / BCF_{fish}$	$NOEL_{predator} / BSAF_{fish}$	Committee
		$NOEL_{predator} / BCF_{fish} \times K_{oc}$ (or K_{ow}) $\times f_{oc}$	RIVM

these NOEC values relates to the death, reproduction or growth of one animal species taken from an ecosystem; the quoted effects are considered to have an effect on the continued existence of the population. In order to be able to derive recommended exposure limits for the protection of ecosystems from these NOEC values, specific extrapolation methods are applied. If NOEC values are known for four or more species of organisms, then the method of Aldenberg and Slob comes into consideration (RIVM91b). Otherwise, the method of the United States' Environmental Protection Agency (EPA) is used (EPA84). An NOEC value is then divided by one or more uncertainty factors, depending on the nature and quantity of the toxicological data available. For each of the organisms and environmental compartments referred to above, a recommended exposure limit is calculated. The lowest of these values per environmental compartment is then the ecotoxicological recommended exposure limit for the ecosystem concerned. Implementation of the ecotoxicological recommended exposure limit should protect at least 95% of the species, which agrees with the MTR (Maximally Acceptable Risk; TK89) laid down by the government. In section 4.4, the recommended exposure limits referred to are derived.

Given the many quantities with which calculations have to be performed, in table 5 the Committee provides a schematic overview of the derivation procedures for the aquatic ecosystem (except for the uncertainty factors used). For further details, the Committee refers the reader to the 'Guidance document' of the RIVM (RIVM92a), and to the report 'Integral Norm-setting for Substances' of another Committee of the Health Council (GR95). The method used for 'aquatic organisms in water' is the simplest: the recommended exposure limit can be directly determined from the NOECs obtained in experiment. For birds and mammals that are indirectly exposed, namely via their food, to dioxin-like substances in water, an additional step is required. In that particular case, the NOECs relate to the concentrations in the food and must therefore be converted to NOECs in water with the help of so-called bioconcentration factors

(BCFs); these express the relationship between the relevant concentrations in food and those in water.

Determining recommended exposure limits for sediment also proceeds in stages, due to the lack of toxicity data for this compartment. As table 5 reveals, there are two possible approaches. First, in the case of aquatic organisms we can start with an 'internal NOEC' (the concentration in the organism at which no effect is observed) and divide this value by an accumulation factor; this factor is designated by the acronym BSAF (Biota to Soil Accumulation Factor), and indicates the relationship between the concentration in the organism and that in the sediment. A second possibility is to select the corresponding NOEC for the compartment 'water' as our point of departure and then to apply a partition coefficient to it. Partition coefficients describe the distribution of a substance between two compartments in a state of equilibrium. The distribution between sediment and pore water appears to be closely related to the organic carbon content in the sediment. That is why the relevant coefficients are usually normalised on that content (K_{oc}). The factor f_{oc} is the fraction of organic carbon in the sediment.

In deriving recommended exposure limits, the RIVM has followed the latter procedure (RIVM93). The Committee, however, prefers the first method. According to the Committee, the quantities that arise in the first method in respect of very hydrophobic substances such as dioxins can be measured, relatively speaking, more reliably. Similarly, the recommended exposure limits for birds and mammals can, in the Committee's opinion, be derived more effectively with the help of BSAF values than via the combined use of BCF and K_{oc} values.

If we are dealing with the environmental compartment 'soil', then the recommended exposure limits for soil organisms are calculated directly from the relevant NOECs or, if these data are lacking, the recommended exposure limit for soil is assumed to be equal to that for sediment. For birds and mammals, the values are derived from the NOECs that have been obtained - which are valid for the concentration in the food (often worms) - and from the BSAF for worms.

In section 4.5, the Committee investigates the measured concentrations of different dioxin-like compounds in the relevant environmental compartments, while in section 4.6 it examines the (im)possibility of pronouncing judgment on the risks to ecosystems within the Netherlands.

In section 4.7, the Committee evaluates the data on farm animals.

4.2 Partition coefficients and accumulation

4.2.1 Partition coefficients

As has already been stated in section 4.1, partition coefficients can play a part in the derivation of ecotoxicological recommended exposure limits. The Committee now offers some technical details. It has noted that these coefficients are usually normalised on the organic carbon content in the soil or the sediment (K_{oc}). The K_{oc} can be roughly calculated from the so-called K_{ow} (the octanol-water-partition coefficient). Reported K_{ow} values for 2,3,7,8-TCDD vary between $2.4x10^5$ and $1.3x10^7$, and K_{oc} values between $1.2x10^3$ and $3.9x10^7$ (McK92). Wide variations of this kind are also encountered in the case of other congeners; the values can, in addition, cover entirely different ranges. In determining recommended exposure limits for the sediment, the RIVM uses the $\log K_{ow}$ value of 6.8 for 2,3,7,8-TCDD; and assumes that this value is valid for all congeners.

4.2.2 Bioaccumulation in the aquatic ecosystem

Aquatic organisms appear, in general, to absorb between 0% and 50% of the PCDDs and PCDFs from their food (Opp90). For PCBs, this fraction is approximately 50% (RIVM92a). For small organisms that live permanently in the water, the direct intake is often more important than the indirect intake via the gastro-intestinal tract. In organisms that forage in the sediment, intake via the ingestion of particles also plays a part. Predators within the aquatic ecosystem, especially mammals and waterfowl, accumulate PCDDs, PCDFs and PCBs via their food. Reptiles, amphibians and insects do so as well, but are not being considered by the Committee due to the lack of satisfactory data on this accumulation.

Accumulation from water

In this case, most of the data relate to the bioaccumulation in freshwater fish of PCDDs and PCDFs from the water. These substances accumulate, above all, in the liver and in the fatty and muscular tissue of the fishes. Experimentally determined bioconcentration factors (BCFs) lie, in this case, between 100 and 86,000 l/kg (RIVM93). These values are of the same order of magnitude as the BCF values for PCBs having similar K_{ow} values. In the case of PCBs, it is also observable that strong bioaccumulation arises when chlorination increases. This tendency needs to be modified slightly for PCDDs and PCDFs. Should chlorination increase, the position of

the chlorine atoms in the molecule is also decisive for the intake and the biotransformation (Opp90, Sij93).

For the derivation of the recommended exposure limits for 2,3,7,8-TCDD, the Committee is using the average BCF value of 20,000 l/kg taken from the literature overview of Loonen (Loo94a).

Accumulation from sediment

Sediment is an important source of exposure for organisms that live in direct contact with it, such as worms and mosquito larvae. In organisms that do not have any direct contact with the sediment, such as many types of fish, a diminished bioavailability of hydrophobic compounds is usually observed. The accumulation in organisms of substances from the sediment is, as remarked in section 4.1, usually described in terms of a BSAF value. Loonen compared the BSAF values for sediment after contact times of, respectively, three weeks and 21 months (Loo94b). For an oligochaete worm, the BSAF for 2,3,7,8-TCDD decreased from 1.6 to 1.1 (kg of organic carbon per kg of fat weight). For OCDD, the BSAF fell from 0.07 to 0.03. This phenomenon is called ‘ageing’. Apart from ageing, the bioavailability of dioxin-like compounds in the field can differ markedly from that in the laboratory, depending on the characteristics of the soil or the sediment, the presence of other substances, and the behaviour of the organisms (Bel95).

The BSAF for 2,3,7,8-TCDD in the carp is 0.71 kg of dry matter in the sediment per kg of the fresh weight. Converted in the light of the organic carbon content in the sediment and the fat content in the fish, the BSAF is 0.14. This value shows close agreement with the BSAF for 2,3,7,8-TCDD found in guppies of 0.155 (kg of organic carbon per kg of fat weight). For all the more highly chlorinated PCDDs and PCDFs, the BSAF is considerably lower (Loo94a). For the derivation of recommended exposure limits for 2,3,7,8-TCDD, the Committee is using a BSAF value of 0.155.

4.2.3 Bioaccumulation in the terrestrial ecosystem

Data on accumulation in terrestrial organisms is scarce (RIVM93). The dioxin-like substances present in the soil are very probably taken in to a limited extent by plants and, in as far as this occurs, transported to a minor extent to the above-ground plant parts. However, deposition from the air and evaporation from the soil play a part as well. BSAF values for the accumulation of 2,3,7,8-TCDD in earthworms vary from 0.2 to 4.7 for a normalised soil containing 10% organic material. In a recently published study of the intake and accumulation of PCBs from contaminated soil from the

Volgermeerpolder, it appeared that the PCB body burden in earthworms was no higher than the contents in the ground (Bel94).

4.3 Toxic effects

In the following sections, the Committee sums up the results of a series of studies of the harmful effects of exposure to dioxin-like compounds. In many cases, these results relate to effects on the survival, reproduction and growth of organisms. As a general rule, also when other toxic substances are being studied, these are the aspects that tend to be subject to examination, because the continued existence of populations (and ecosystems) can be associated with them directly (GR93, RIVM92b).

4.3.1 Aquatic ecosystems

Possible consequences of exposure to PCDDs, PCDFs and PCBs have been described for invertebrates, fish, fish-eating birds, and marine and freshwater mammals. These consequences include: death, restricted growth and reproductive capacity, porphyria, and changes in biochemical characteristics such as cytochrome P4501A1 enzyme activity (EROD), thyroid hormone concentrations in the plasma, and vitamin A contents in the plasma and the liver (Ber94a, Bos94, Bos95, Bro89, Bro91, Rei86).

Invertebrates

Data on the toxicity of dioxins for invertebrates are scarce and inadequate. For the worm, dioxin-like substances are 1000 times more toxic than aspecifically (narcotically) active substances such as chlorobenzenes (Bel94).

Fish

Compared to invertebrates, fish are very sensitive to the effect of exposure to PCDDs, PCDFs and PCBs. They are exposed through intake from water, food and sediment. Fish eggs are indirectly exposed via the mother, and directly through contact with water and soil. Young fish display a wide range of effects following exposure: induction of cytochrome P4501A1, decrease in food consumption and body weight, abnormalities in the skin and lymph glands, and death. Even earlier stages in the life cycle display oedema of the yolk-sac, haemorrhages, and death (Sij96). Of the tested species, the rainbow trout appears to be the most sensitive to the toxic effect of PCDDs and PCDFs; the most sensitive life phase is the early development. In a flow experiment on rainbow trout, the lowest concentration of 2,3,7,8-TCDD in water that

had an effect on the survival of fish larvae was 0.038 ng/l (Meh88). On the basis of this result, the RIVM derived an NOEC of 0.012 ng/l (RIVM93). The internal concentration corresponding to this figure of 0.038 ng per litre is 980 ng per kg of fish. Given the same ratio between the concentration in water and the concentration in fish, the derived NOEC for water should correspond to an internal NOEC for fish of 309 ng of 2,3,7,8-TCDD per kg.

According to an overview by Sijm and Opperhuizen, the experimentally measured internal concentrations of 2,3,7,8-TCDD at which death arises vary from 54 ng per kg of fish to 2 042 000 ng per kg of fish (Sij96). The lower value is derived from research on salmon; for the rainbow trout, the following values were obtained in different studies: 3000, 5000, 10000 and 25000 ng per kg of fish. For the carp, a fish often found in the Netherlands, the sensitivity appears to be roughly equal to that of the rainbow trout.

For the eggs of the rainbow trout, the lethal concentration amounted to 421 ng/kg of eggs. In this case, the exposure was via injection, but direct exposure of the eggs via the water revealed a comparable value (Wal92). The Committee notes in parenthesis that, in these experiments, TEF values were also established (Wal91). The TEF values discovered for PCDDs and PCDFs agreed closely with those measured in liver cells of the rat. The values for PCBs seemed to turn out 14 to 86 times lower than those found in the rat.

Mammals

Seals that were fed with fish contaminated with PCBs reproduced with diminished success, and had abnormal thyroid hormone and vitamin A contents (Ahl92a, Bro89, Bro91). In another study, seals were fed over a long period either with highly contaminated fish from the Baltic Sea or with fish with a much lower level of contamination from the Atlantic Ocean. The animals that ate the highly contaminated fish displayed abnormalities in the immune system (Ros95, Swa95).

A great deal of research has been done on the mink, a carnivore related to the otter. High PCB concentrations among wild otters and mink can be associated with the fact that populations are becoming smaller (Ahl92a). The mink is often used as a model for fish-eating mammals. The results of exposure to dioxin-like substances are, among other things, a reduced survival rate among the young, and an increase in the weight of the liver. The sensitivity of the mink is somewhere between that of the guinea pig and the rat. Lethality experiments have revealed that mink are approximately five times more sensitive than rats to the acute toxic effect of 2,3,7,8-TCDD (Cou94). The EC_{50} (the concentration at which the effect under consideration occurs in 50% of the animals) for growth inhibition in young animals was $0.1x10^3$ ng per kg of food per day

(Aul88, Loo94b). Recently published research results have brought to light a link between exposure and the birth weight of the offspring (Til96). In these studies, the mink were fed with fish from a contaminated area. The fish contained a mixture of PCDDs, PCDFs and PCBs. There were three exposure levels: 10%, 20% and 40% of contaminated fish in the food of the mink. In the case of the lowest dose, which corresponded to 12.6 ng TEQ_{TOTAL} per kg of fish, an effect was still observable.

Fish-eating birds

Exposure of cormorants to PCDDs, PCDFs and PCBs, measured with the help of increased enzyme activity, yolk-sac concentrations, and *in vivo* EROD induction in tissue extracts, correlated negatively with hatching success and positively with developmental disturbances (Ahl92a, Ber94b). Dioxin-like PCBs play an important part in the Ah receptor-mediated effects in fish-eating birds; more than 50% of the TEQ_{TOTAL} in tissue can be originated in these compounds. In common terns, that figure is as high as 90% (Bos94). These birds are more sensitive than cormorants, herring gulls and black-headed gulls. Embryonal exposure to mixtures of polyhalogenated hydrocarbons had a negative effect on the egg volume, the duration of the incubation period and the EROD activity in the liver of offspring of common terns in the Netherlands and Belgium (Bos95). An LOEC for the induction of cytochrome P4501A1 activity in the livers of common terns (which were raised in the laboratory) of 46 ng TEQ_{TOTAL} per kg of fish was found. For naturally-raised common terns, a significant induction of cytochrome P4501A1 activity was determined at 16 ng TEQ_{TOTAL} per kg in the yolk-sac and 40 ng TEQ_{TOTAL} per kg of fish in the food. At this concentration level, the hatching period of the birds also took (significantly) longer (2 to 3 days). A NOEC of 20-30 ng TEQ_{TOTAL} per kg of fish was proposed (Bos95).

4.3.2 Terrestrial ecosystems

Only a limited amount of data is available regarding the toxicity of 2,3,7,8-TCDD (RIVM93). From a long-term field experiment (You87) in which a large number of animal species (insects, reptiles, birds and mammals) and plant species were investigated, it can be concluded that no effects arise in respect of 2,3,7,8-TCDD contents in the soil up to 1500 ng per kg of dry matter (RIVM93). One difficulty is that the bioavailability of 2,3,7,8-TCDD in the soil is being estimated very differently.

In pot tests on maize and beans, no observable toxic effect arose in the presence of 2,3,7,8-TCDD contents of 1 to 750 ng per kg of dry matter (Fac86).

For the earthworm *Allolobophora caliginosa*, $10x10^6$ ng per kg was fatal. At half this concentration, all the worms remained alive (Rei84).

No reliable data are available for terrestrial predators. In the opinion of the Committee, data for the common tern, the mink (see section 4.3.1) and the rat can be used provisionally. Laboratory experiments on the rat have been carried out with 2,3,7,8-TCDD in the food. From these, an NOEC of 20 ng per kg of food per day can be derived (RIVM93).

4.4 Ecotoxicological recommended exposure limits

In the following paragraphs, the Committee indicates which data from the foregoing sections it considers to be the most suitable for determining ecotoxicological recommended exposure limits, and calculates the required values for the various environmental compartments. Before that, the Committee expatiates further on the toxicity of the various congeners. As the Committee stated in section 2.2, it is, in principle, appropriate to use separate ECOTEFs for different groups of animals. However, a reliable use of these ECOTEFs in the derivation of recommended exposure limits is, in the Committee's opinion, still impossible: on the one hand, the ECOTEF values discovered for several species diverge too much for this to be feasible; on the other, these values differ depending on the effect under consideration. In addition, there is another problem. ECOTEF values have, it is true, been published for fish, birds and mammals, but these are not related to concentrations in the environmental compartments. If we want to be able to use the relevant ECOTEFs to derive recommended exposure limits, then the accumulation from 'water' and 'sediment' to 'fish' must be determined for each of the congeners involved. The ECOTEF values for birds and mammals apply to concentrations in the food. Here also, conversions to the compartments 'water', 'sediment' or 'soil' must be made for the individual congeners. The data (BSAF or BCF values) required to determine these values are, in most cases, completely lacking. In the Criteria Document 'Dioxins' (RIVM93), the RIVM expresses recommended exposure limits for the various environmental compartments in the form of TEQs derived with the assistance of human TEF values, and also omits the conversions just referred to. The Committee considers this procedure to be incorrect, and gives recommended exposure limits only for 2,3,7,8-TCDD. This — in the Committee's view — unavoidable limitation has yet another striking aspect. It is not 2,3,7,8-TCDD, but rather a certain number of non-ortho and mono-ortho PCBs, that make by far the largest contribution to the exposure of organisms in aquatic ecosystems in the Netherlands (Bos95). The Committee therefore pleads that the RIVM, in its criteria document on 'PCBs' (not yet published), pays special attention to these congeners in its ecotoxicological risk evaluation.

Table 6 Recommended exposure limits for the aquatic ecosystem and the data used for the derivation.

	water	sediment
aquatic organisms	NOEC= 0.012 ng/l EPA=10 *recommended exposure limit = 0.0012 ng 2,3,7,8-TCDD per litre of water* RIVM: same value	$NOEC_{(internal)}$ = 309 ng 2,3,7,8-TCDD per kg of fish EPA=10 BSAF= 0.155 *recommended exposure limit = 200 ng 2,3,7,8-TCDD per kg of dry matter* RIVM NOEC = 0.012 ng TEQ/l EPA = 10 K_p= 315000 l/kg *recommended exposure limit = 378 ng TEQ per kg of dry matter*
birds and mammals	NOEC (common tern) = 20 ng TEQ_{TOTAL}/kg of fish[a] LOEC (mink) = 12.6 ng TEQ_{TOTAL}/kg of fish[a] NOEC (rat) = 20 ng 2,3,7,8-TCDD/kg of food EPA = 10 BCF_{fish} = 20000 l/kg *recommended exposure limit = 0.0001 ng 2,3,7,8-TCDD per litre of water*	NOEC (common tern) = 20 ng TEQ/kg of fish[a] LOEC (mink) = 12.6 ng TEQ/kg of fish[a] NOEC (rat) = 20 ng 2,3,7,8-TCDD/kg of food EPA = 10 BSAF= 0.155 *recommended exposure limit = 13 ng 2,3,7,8-TCDD/kg of dry matter*

[a] The TEQ value stated here was calculated with the help of mammalian TEFs (see chapter 2). It serves only for purposes of comparison with the concentration in fish in the Dutch environment, which is calculated using the same TEF values.

4.4.1 *Recommended exposure limits for aquatic ecosystems*

Table 6 contains the recommended exposure limits for the aquatic ecosystem, as well as the data used in the derivation of the values. In the absence of adequate toxicity data for the various species of organisms, use is made, in respect of all the recommended exposure limits, of the EPA's extrapolation method. Given that NOECs for chronic exposure were available in every case, an extrapolation factor of 10 has been used. The recommended exposure limits have been derived using the formulae from table 5.

For the sake of completeness, the Committee has also indicated which figures the RIVM has used in the alternative derivation methods, and the results to which these lead.

Recommended exposure limits for aquatic organisms

In order to determine a recommended exposure limit for aquatic organisms in water, the Committee considers the NOEC for 2,3,7,8-TCDD estimated by the RIVM of

0.012 ng/l on the basis of a test using the rainbow trout to be the most relevant (Meh88, RIVM93). Division by the extrapolation factor of 10 gives a recommended exposure limit of 0.0012 ng of 2,3,7,8-TCDD per litre.

With reference to the recommended exposure limit for sediment, the Committee has already noted in the introduction to this chapter that it finds a derivation carried out on the basis of internal NOECs and BSAF values to be the most justified on scientific grounds. Although the determination of these quantities is also prone to uncertainties, the problems are less serious than those involved in the use of partition coefficients. In the experimental determination of partition coefficients, for example, it is difficult to distinguish between the sediment and water phases. In addition, it is true of hydrophobic substances such as dioxins that their concentration in the water phase - to which the coefficients referred to must be applied (see table 5) - is very far from being homogeneous. This is something that is associated with the poor solubility of these compounds. In addition, part of the dioxins is unavailable due to sorption to food, the test system and the faeces of the organisms under investigation. The internal NOEC that corresponds to the NOEC in water (0.012 ng/l) mentioned above, is 309 ng of 2,3,7,8-TCDD per kg of fish (see 4.3.1). Use of the BSAF 0.155 (section 4.2.2) and of the extrapolation factor of 10 gives a recommended exposure limit for sediment of 200 ng of 2,3,7,8-TCDD per kg of dry matter. In as far as data for other congeners are available, this points, in the Committee's opinion, towards higher recommended exposure limits; accordingly, the BSAF values are usually lower (up to a factor of 100) (Loo94b). Indeed, the total spread in values covers three to four orders of magnitude (Bel95).

The Committee would like to add a note to this derivation. In this case, it has used as its starting-point an *internal* NOEC for young fish instead of the lower *observable internal* NOEC for eggs. Because data on the relationship between the concentration in the mother fish and in the eggs are lacking, the Committee cannot use this figure as a basis for deriving recommended exposure limits for dioxin-like substances.

Recommended exposure limits for birds and mammals

Although there is a NOEC for a fish-eating mammal and a LOEC for a fish-eating bird (the mink and the tern respectively, see section 4.3) these have been expressed in TEQ_{TOTAL} per kilogram of food. The Committee cannot, for the reason stated above, use this value for the derivation of recommended exposure limits for water and sediment. However, the NOEL of 2,3,7,8-TCDD for the rat can be used: this is 20 ng/kg of food. The recommended exposure limit for 'water' is arrived at by applying to this the BCF 20000 l/kg (see section 4.2.2) and the extrapolation factor 10, giving a result of 0.0001 ng 2,3,7,8-TCDD per litre.

In the case of the environmental compartment ‘sediment’, the Committee thinks that the value 20 ng of 2,3,7,8-TCDD per kg of food must be divided by the BSAF of 0.155 and the extrapolation factor 10, so that the recommended exposure limit is 13 ng of 2,3,7,8-TCDD per kg of dry matter.

Ecotoxicological recommended exposure limit

Of the recommended exposure limits just derived, those for birds and mammals turn out to be lower than those for aquatic organisms. The ecotoxicological recommended exposure limit for aquatic ecosystems is therefore 0.0001 ng of 2,3,7,8-TCDD per litre for water, and 13 ng of 2,3,7,8-TCDD per kg for sediment. If we compare the recommendations of the Committee and those of the RIVM (table 6), it can be established that the various recommended exposure limits differ from one another only slightly, certainly if we take into account the uncertainties involved. An important difference is that the Committee is restricting itself to recommended exposure limits for 2,3,7,8-TCDD.

4.4.2 Recommended exposure limits for terrestrial ecosystems

Recommended exposure limits for soil organisms

Toxicity data for soil organisms are scarce. For the time being, the Committee has a preference for making the recommended exposure limit for these organisms equal to that for aquatic organisms, a usual procedure under such circumstances (RIVM92b). This value is therefore 200 ng of 2,3,7,8-TCDD per kg of dry matter.

The RIVM has, with the help of data for the earthworm, derived a recommended exposure limit of 0.5×10^6 ng TEQ_{TOTAL} per kg of soil (RIVM93). However, the Committee does not consider the earthworm to be a very suitable species upon which to base a recommended exposure limit for the soil. After all, in aquatic ecosystems, this species seems to be relatively insensitive.

Recommended exposure limit for birds and mammals

For these predators also, we have little or no toxicological information. The Committee is taking the experimentally determined NOEC for the rat as its starting-point. For this mammal, the NOEC is, as remarked earlier, 20 ng of 2,3,7,8-TCDD per kg of food. Dividing this value by the BSAF for worms and the extrapolation factor already used gives the recommended exposure limit that we are seeking. In section 4.2.3., it is stated that the BSAF values referred to vary from 0.2 to 4.7. The Committee is selecting the

value 1, and thus arrives at a recommended exposure limit of 2 ng of 2,3,7,8-TCDD per kg of dry matter.

The RIVM has, on the basis of data relating to the rat, derived a recommended exposure limit of 2 ng TEQ per kg.

Ecotoxicological recommended exposure limit

Because the recommended exposure limit for birds and mammals is lower than that for soil organisms, the Committee proposes an ecotoxicological recommended exposure limit for the protection of terrestrial ecosystems of 2 ng of 2,3,7,8-TCDD per kg of dry matter in the soil.

4.5 Concentrations in the environment

In the previous section, the Committee stated its objections to the reported TEQ values. Here it is confining itself to concentrations of 2,3,7,8-TCDD.

4.5.1 Concentrations in the aquatic environment

PCDDs, PCDFs and dioxin-like PCBs in surface water in the Netherlands originate, to a significant extent, outside the country. They are brought into the Netherlands through the deposition of contaminated particles and via the Great Rivers (RIVM93, RIVM95a). Point sources also contribute to the burden (Tur86).

In a sedimentation area of the Rhine, the Ketelmeer, dioxin-like compounds were measured in sedimentary layers of varying dates (RIVM93). In layers that were deposited at the beginning of the 1940s, the contents of 2,3,7,8-TCDD and of most of the other dioxins are very low, or are even below the detection limit (10 ng per kg). Exceptions to this are the more highly chlorinated PCDDs and PCDFs. Sedimentary layers from the 1960s and 1970s show the highest contents. The highest measured concentration of 2,3,7,8-TCDD in this period is 450 ng per kg. The contents in recently deposited sediment agree, for the most part, with those from the beginning of the 1940s. It is only for dioxin-like PCBs that the concentration levels are still increasing.

In the coastal waters, the concentration of dioxins and dioxin-like PCBs appears to be rising again following a initial fall. In the surface water, the PCB concentrations were, in general, higher in 1988 and 1989 than in the period 1990-1993. They have remained constant over the past few years.

4.5.2 *Concentrations in the terrestrial environment*

The average background concentration of dioxins in air in urban industrial areas is anything up to twice as high as that in country areas (RIVM93). In the Netherlands, few measurements have been made of atmospheric concentrations of dioxin-like PCBs.

The average background concentration of dioxins in the soil in the Netherlands is only available in the form of $TEQ_{DIOXINS}$, and amounts to 2-5 ng $TEQ_{DIOXINS}$ per kg of dry matter (RIVM93). The PCB burden in the topmost 5 cm of Netherlands soil is estimated at 23 tons. This agrees with an average concentration of $12.5x10^3$ ng of PCB per kg of dry matter (RIVM95a).

4.6 Risks for ecosystems

In this chapter, the Committee has pointed out three sources of difficulty: there are still no usable TEF values for calculating the concentrations of dioxin-like compounds in the different groups of organisms, there are no BCF and BSAF values for the translation to the environmental compartments of concentrations of the separate congeners in organisms, and the concentrations of 2,3,7,8-TCDD are of limited importance due to the fact that they make only a small contribution to the total exposure in the aquatic ecosystems in the Netherlands. These factors stand in the way of a complete risk evaluation. However, on the basis of the data available at the present time, some indications can be given regarding the degree to which ecosystems can suffer damage through the present exposure levels in the Netherlands. Thus, the TEQ_{TOTAL} content in fish from the Haringvliet - in this case the Committee considers the TEQ value to be a suitable quantity, relating as it does to a value in the food - appears to amount to 40 ng per kg (Bos95). This value is twice as large as the NOEC mentioned above for the common tern, and more than three times as large as the LOEC for the mink.

4.7 Risk evaluation for farm animals

4.7.1 *Introduction*

Being terrestrial organisms, farm animals are exposed to concentrations of PCDDs, PCDFs and dioxin-like PCBs. In this case, though, the risk evaluation does not just relate to the animals themselves. They supply milk fats and other animal fats that - as the Committee has already pointed out in chapter 3 - constitute the most important

source of exposure for man. When deriving recommended exposure limits for these animals, therefore, we will also have to take this latter point into account.

4.7.2 *Bioaccumulation*

Accumulation takes place, above all, in fatty tissue and in the liver. In lactating cows, excretion in the milk can be more considerable than the metabolic clearance. In the case of exposure to 500 ng of 2,3,7,8-TCDD per kg of food, concentration factors of 0.18 for milk and 1.56 for cream have been calculated. The elimination half-life in milk fat varies from 30 to 110 days depending on the congener (RIVM92a). From Dutch nutritional research (RIVM91a), it appeared that more than half the total exposure ($TEQ_{DIOXINS}$) of the population via the diet over a period of 70 years is attributed to foodstuffs such as milk, butter, cheese and beef fat. Contents in animal products in the Netherlands have been investigated on many occasions (RIVM95b).

4.7.3 *Effect on the health of farm animals*

Various incidents have been described in which farm animals were exposed to PCDDs, PCDFs and PCBs (McC89). Following one such exposure in 1947, cows suffered hard swellings of the skin (hyperkeratosis) on the head, neck and shoulders. This was accompanied by lacrimation, salivation, diarrhoea and polyuria. These symptoms were collectively named ‘X disease’. It appeared that this disease could also occur in sheep and pigs, for example as a result of the contamination of fodder with lubricants, through the use of waste oil products as a solvent for insecticides in the treatment of parasites, and from the licking up of wood preservatives contaminated with PCDDs and PCDFs. In Michigan, in 1973 and 1974, thousands of cows, pigs, sheep and chickens had to be slaughtered on account of X disease-like symptoms, following the inadvertent introduction of polybromine biphenyls into fodder. In addition, considerable quantities of milk, cheese and eggs had to be destroyed. No data on reproduction toxicity are available.

Chickens

In 1957, in the south-east of the United States, a very considerable number of chickens died because their feed (fat) had become contaminated with PCDDs, especially HxCDD, while it was being prepared from animal skins. A mixture of tri- and tetrachloride-substituted dioxins and furans in the feed led, at an intake of 1.8 ng per kg of body weight per day, to chicken oedema and death (Fli72a, Fli72b). The

incidence of heart abnormalities in embryos doubled after the administration of a single dose of TCDD of 0.3 ng per egg (Che81, Loo93).

Cattle

Studies have been carried out on cows that were orally exposed, especially to the persistent 2,3,7,8-substituted dioxins (RIVM93). In this case, the bioavailability was 30%-50% for tetra-, penta-, and hexacongeners, and 1%-3% for hepta- and octacongeners. The substances were added directly to the fodder, for example in olive oil. In soil and fly ash, the availability is 0.5%-10% for the total group of dioxins. Enzyme induction in the animals arose after a single oral dose of 50 ng of 2,3,7,8-TCDD per kg of body weight (Jon89). Much research has been done on cattle using so-called technically pure or analytically pure pentachlorophenol (PCP) contaminated to a greater or lesser extent with PCDDs and PCDFs. In a fodder test, young cattle displayed a reduction in growth rate after three weeks at a dose of $15x10^6$ - $20x10^6$ ng of t-PCP per kg of body weight per day. In the case of a 6-week exposure to a dose of $1x10^6$ ng t-PCP per kg of body weight per day, an increased liver weight and a reduced thymus weight were observed (RIVM93).

Sheep and lambs

Studies have been carried out on sheep that were exposed orally (via cattle cakes) to 131 ng $TEQ_{DIOXINS}$ per day for eight days (RIVM94). The concentration of these substances in the fat of lambs at birth was 30% of that in the ewes. The TEQ_{TOTAL} value was determined, above all, by a high concentration of 2,3,7,8-TCDD. The degree of placental transfer of PCDDs and PCDFs decreases as the degree of substitution increases. Quite the opposite is true of transfer via breast-feeding, which has much greater significance in determining the final body burden of the lambs.

4.7.4 Recommended exposure limits

Unfortunately, no data from chronic toxicity experiments with PCDDs, PCDFs and dioxin-like PCBs are available that would allow the derivation of recommended exposure limits.

Bioaccumulation in man has, up to now, formed the basis for recommended exposure limits for the products of farm animals and, in its turn, for farm animals themselves. The Committee is not giving any recommended exposure limits for separate agricultural products.

4.7.5 Exposure

Grazing farm animals, such as cows and sheep, are exposed to dioxin-like substances from the soil and grass. The contribution via air and water is negligible. However, the chance does exist, in the neighbourhood of a local source, of an increased exposure via deposition from the air onto the soil and grass. This can lead to increased contents in meat and milk. In addition, it has been shown that, in the winter, exposure via contaminated ensilage occurs. In the past, concentrations up to a maximum of 0.012 ng $TEQ_{DIOXINS}$ per gram of milk fat have been found in cows that were grazing in the vicinity of a waste incineration plant, while Dutch cow's milk in general has a background content of 0.0008 to 0.0025 ng $TEQ_{DIOXINS}$ per gram of milk fat (RIVM92c).

4.7.6 Risks

In the Netherlands, diseases have been reported in farm animals that live in the vicinity of waste incineration plants, but a causal link with exposure to dioxin-like substances could not be demonstrated.

Chapter 5

Response to the Minister's questions

5.1 Health-based and ecotoxicological recommended exposure limits and the evaluation of health risks

This Criteria Document evaluates, for the Dutch exposure situation, the risks to man and the ecosystem by means of a comparison with, respectively, the TDI and the MTRs for ecosystems and predators that are derived within the document. This is the first time that a Criteria Document has taken into account the possible consequences of secondary poisoning. Is the Health Council able to support the derived TDI and MTR levels and the conclusions of the evaluation?

The present data suggest that, both in carnivores and primates, a dose of 0.1 ng of 2,3,7,8-TCDD per kg per day is an LOAEL, but certainly not an NOAEL, for some of the observed effects (section 3.3.1). This applies to biochemical parameters, but also to more clearly adverse effects such as endometriosis. On the basis of laboratory animal research, the Committee derives an NOAEL of 0.05 ng of 2,3,7,8-TCDD per kg of body weight per day. It is applying to it the safety factors 5 (for the interspecies variation, which here means the variation between monkey and man) and 10 (for the intraspecies variation), which means that the health-based recommended exposure limit is 1 pg of 2,3,7,8-TCDD per kg of body weight per day. Given that their effects are similar, the Committee considers this recommended exposure limit to be applicable also to exposure to mixtures of different dioxin-like compounds, expressed in toxic equivalents (TEQ_{TOTAL}). This value is 10 times smaller than the recommended exposure limit for the intake (TDI) cited in RIVM93.

The reason for the difference is that the Committee is basing itself on other, in part more recent, laboratory animal research. It also thinks that data on the effects of Dutch infants can be used for the risk evaluation, and that this supports its proposed health-based recommended exposure limit (section 3.3.2). The effects referred to occurred in breast-fed children whose mothers were exposed to the present background levels of dioxin-like compounds. What is more, we are dealing here with subtle changes in the most highly exposed group of infants. They display a suboptimal neurological development, a slight retardation in psychomotor development, and changes in the thyroid hormone status and immunological functions. The Committee emphasises that the differences in effects between infants undergoing high and low levels of exposure fall within the range of the clinical reference values. With respect to the long-term relevance to health of the measured differences, the Committee's opinion is that, given the data currently available, no clear verdict can be delivered. However, for safety's sake, the Committee considers such effects to be undesirable.

Due to the limited amount of reliable data available on different species, the Committee is using, for the derivation of the ecotoxicological recommended exposure limits, fixed extrapolation factors, taking as a starting-point data for 2,3,7,8-TCDD for a sensitive species.

The recommended exposure limits for ecosystems derived by the Committee cannot be readily compared with those in RIVM93, given that the Committee is only issuing recommended exposure limits for 2,3,7,8-TCDD (section 4.4). There are various reasons for this. The published ECOTEF values for different groups differ too much for these to be used. Measured LOEC and NOEC values for birds and mammals are concentrations in food, and, for the translation to compartments to be made, congener-specific BCF and BSAF values are required that are only available for 2,3,7,8-TCDD. The Committee also notes that it is not 2,3,7,8-TCDD that makes the biggest contribution to the exposure of organisms in the Netherlands, but a number of non- and mono-ortho PCBs. To protect predators, such as fish-eating birds, the Committee proposes a recommended exposure limit for the aquatic environment of 0.0001 ng of 2,3,7,8-TCDD per litre of water. Since the recommended exposure limit for birds and mammals is lower than that for aquatic organisms, the limit is normative for the aquatic ecosystem. As the recommended exposure limit for sediment, the Committee proposes 2 ng of 2,3,7,8-TCDD per kg of dry matter (section 4.4.1).

Of the recommended exposure limits for terrestrial ecosystems derived by the Committee (section 4.4.2), the value for soil organisms differs considerably from the value proposed in RIVM93 (a factor of 2500 smaller). The Committee prefers to make the value for soil organisms equal to that for water organisms in sediment: 200 ng of 2,3,7,8-TCDD per kg of dry matter. To lay down a value for birds and mammals, the

Committee is using data from the rat (RIVM93). This data lead to a recommended exposure limit of 2 ng of 2,3,7,8-TCDD per kg of dry matter in the soil (section 4.4.2). The last value is lower than that for soil organisms and, by virtue of that fact, is normative for the terrestrial ecosystem.

In the aquatic ecosystem, the recommended exposure limit (which agrees with the MTR level) for sediment is locally exceeded. In general, it can be said that, especially in predators that are dependent on the aquatic environment, effects can be expected given the present level of exposure at certain locations. The process of bioaccumulation in the aquatic ecosystem is complicated by the presence of suspended and dissolved organic matter and sediment that influences the bioavailability of PCDDs, PCDFs and dioxin-like PCBs through sorption and desorption. The Committee also points out that the bioavailability of dioxin-like substances decreases after a long contact time between these substances and sediment. Due to this phenomenon of 'ageing', the bioavailability in the field is almost always lower than in laboratory experiments.

Given the non-availability of concentrations 2,3,7,8-TCDD in the soil, it is impossible to deliver a verdict on whether the recommended exposure limits for soil organisms, birds and animals is being exceeded, whether locally or not.

5.2 Is 2,3,7,8-TCDD a non-genotoxic carcinogen?

In the evaluation, the starting-point for man was the TDI proposed by the WHO/EURO in 1990 of 10 pg of 2,3,7,8-TCDD/kg of body weight, and it was established that the TEF principle can be used to evaluate mixtures. What this comes down to in practice is a TDI of 10 pg TEQ/kg of body weight. This TDI is based on chronic toxicity and carcinogenicity experiments in laboratory animals, and takes into account differences in kinetics between laboratory animals and man. The basic assumption in this case is that the carcinogenic properties of 2,3,7,8-TCDD do not rest on a genotoxic working mechanism, and that an effect-threshold approach can be used as a starting-point. Does the Health Council share the opinion that 2,3,7,8-TCDD must be considered a non-genotoxic carcinogen?

The Committee associates itself with the verdict of the Committee on Carcinogenic Risk Assessment. It concludes from the available data that 2,3,7,8-TCDD is carcinogenic in laboratory test animals. Epidemiological data on exposure to 2,3,7,8-TCDD are conflicting, and does not provide a conclusive answer as to whether this substance is carcinogenic in man. Longer-term observation of the relevant cohorts may perhaps offer more clarity in this matter. For the time being, therefore, an estimate of the chance of cancer arising as a result of exposure to 2,3,7,8-TCDD must be based on laboratory animal data (see Annex A.4).

The Committee concludes that the carcinogenic properties of 2,3,7,8-TCDD rest on a non-genotoxic effect (Annex A.3). It therefore recommends deducing a recommended exposure limit on the basis of an NOAEL, taking safety factors into consideration. As the basis for determining this kind of recommended exposure limit, the Committee finds the results of the research of Kociba and colleagues (Koc78) to be the most suitable, provided that due account is taken of the results of the re-evaluation of that study (Annex A.4.1).

5.3 Perinatal exposure

Research results that became known after the Criteria Document was completed, such as the Netherlands maternal milk study referred to above, and the USEPA evaluation, cause us to suspect that developmental disturbances resulting from perinatal exposure are important for the risk evaluation of dioxins. What is the opinion of the Health Council on this subject? Is it possible, in the Council's opinion, to evaluate which is the more critical exposure in this respect: prenatal (in utero) exposure or postnatal exposure via the maternal milk? Can the Council indicate whether this fact has consequences for the setting of the exposure norm (TDI)?

The Committee does indeed consider these research results to be of great importance for laying down a health-based recommended exposure limit and a TDI. In particular, it attaches great value to the results of the study carried out among infants in Rotterdam and Groningen (section 3.2.5). From this study, it is true, it emerges that perinatal exposure to PCDDs, PCDFs and PCBs is not associated with serious, clinically relevant disturbances in the development of the new-born, but is accompanied by various other developmental effects - ones that we cannot be sure to be harmless. The possibility that prenatal and postnatal exposure have different effects cannot be firmly established in this case: infants undergoing a high prenatal exposure were also highly exposed postnatally. In addition, exposure data were only available for the breast-fed group. However, the Committee is of the opinion, on the basis of general findings relating to the sensitivity of organisms at different moments in their development, that prenatal exposure is the more critical phase. In addition, the Committee wishes to point out that data on the Yu-cheng accident and the results of laboratory animal research reveal that exposure during this phase can induce almost the full pattern of developmental disturbances referred to (section 3.2.5).

In the Committee's opinion, the exposure of children before and after birth can best be limited by using an exposure norm for the mother and, in its turn, for the whole population. Reduction of breast-feeding is, in the opinion of the Committee, not the right way. Of course, from the Dutch research among infants it is apparent - as has been known for a long time - that breast-fed children do, in some respects, develop

more satisfactorily than formula-fed children; in this connection, the Committee points to such matters as visual recognition, and mental and psychomotor development (section 3.4.2). There is no reason to limit the freedom of parents to choose between breast-feeding and formula-feeding for their infant.

5.4 Should the risk evaluation of dioxins and PCBs be carried out in combination?

In addition to dioxins, PCBs are also present everywhere in the environment. The result of the similarities in physical-chemical behaviour is that there is common exposure to both substance groups. A number of PCBs that, in terms of spatial structure, are comparable to dioxins, also display the same toxic effects as dioxins. This factor is currently taken into account in risk evaluations. For these PCBs, toxic equivalency factors have also been proposed (WHO, 1993). Is the Health Council of the opinion that a risk evaluation of dioxins and PCBs should be conducted in combination? If so, how can this be given expression in the setting of norms?

Dioxin-like PCBs are, depending on the feeding pattern, responsible for at least half the average exposure of the population to dioxin-like compounds expressed in the TEQ (toxic equivalency). The basic assumptions in the use of the concept of 'toxic equivalency factor' (TEF) are that all 2,3,7,8-substituted congeners have a similar working mechanism, and that the individual compounds contribute additively to the toxicity of the mixture (section 2.2). The TEQ_{TOTAL} value is determined by multiplying the concentration of each component of the mixture by the corresponding $TEF_{DIOXINS}$ or TEF_{PCB} value, and then adding up the resulting products. The Committee would like to add a note to this last calculation step. Specific PCBs appear, in relation to dioxins, to have an antagonistic or synergistic — and therefore a non-additive — effect. This is quite separate from possible carcinogenic and behavioural effects that are not mediated by the Ah receptor.

The TEFs for different groups of animal species can vary considerably. The Committee is therefore of the opinion that, in estimating the ecotoxicological risk, separate TEFs (ECOTEFs) must be used for mammals, birds, fish and invertebrates (section 2.2).

The Committee feels that a cohesive risk evaluation of exposure to PCDDs, PCDFs and dioxin-like PCBs is called for. It considers the TEF concept to be a usable uniform instrument in this process.

Rijswijk, 6 August 1996,
for the Committee

(signed)

JAG van de Wiel,	Professor LA Clarenburg,
Secretary	Chairman

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Annexes

Annex **A**

Opinion of the Committee on the Evaluation of the Carcinogenicity of Chemical Substances

A.1 Introduction

The Committee on the Evaluation of the Carcinogenicity of Chemical Substances, hereafter in this annex referred to as the Committee, evaluated the seventeen chlorine substitution polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) - in so far as data were available - for carcinogenic properties. For the purpose of its evaluation the Committee studied the literature used in preparing the Criteria Document on Dioxins (RIVM93) as well as that published subsequently.

A.2 The formation of DNA adducts

The evaluation was confined to a study of the formation of DNA adducts (covalent binding to DNA) of the congeners 2,3,7,8-TCDD and 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (1,2,3,7,8-PCDD).

Recent studies in rats treated with radioactively labelled 2,3,7,8-TCDD or 1,2,3,7,8-PCDD have provided no evidence that DNA adducts can form in the liver or kidneys (Ran88). The most recent study of the formation of DNA adducts of 2,3,7,8-TCDD (Tur90), performed using an exceptionally sensitive detection technique, also had a negative outcome.

From the above, the Committee concluded that 2,3,7,8-TCDD and 1,2,3,7,8-PCDD are not capable of forming DNA adducts.

A.3 Genotoxicity

In vitro tests

2,3,7,8-TCDD was examined in various types of *in vitro* test for mutagenicity. This was done extensively in bacteria, both in the presence and absence of a metabolic activation system (IARC87a, IPCS89). 2,3,7,8-TCDD was found to score positively in the initial tests with bacteria, but negatively in many later tests of this type. In common with the International Agency for Research on Cancer (IARC), the Committee attaches the greatest possible value to the most recent tests, since these were better performed. It therefore endorses the IARC's conclusion that 2,3,7,8-TCDD does not have a mutagenic effect in bacteria.

2,3,7,8-TCDD was also studied extensively for mutagenic properties in eukaryotic test systems. This gave positive scores in two types of test for the detection of gene mutations in yeast cells, namely in a suspension test and the host mediated assay (IPCS89); a positive score was also obtained in a test for the detection of gene mutations in mouse lymphoma cells (IARC87a). The Committee believes that only the last-mentioned test was carried out correctly and that 2,3,7,8-TCDD is rightly referred to as "positive" in this test: the substance caused a significant increase in the number of mutations, and the number of mutations increased with the dose - even up to a factor of twenty (Rog82). With regard to the mechanism involved, the researchers who conducted the test believe that the chemical intercalates the DNA, i.e. that it establishes itself between the two chains making up the DNA molecule. The supposition is that this leads to a "frame shift" mutation, whereby the entire DNA molecule is "read" wrongly. The Committee believes that this explanation is compatible with the negative results of the tests with bacteria, because those tests are not appropriate for demonstrating frame shift mutations.

2,3,7,8-TCDD was not studied for evidence of causing structural chromosome aberrations or sister chromatid exchanges *in vitro* (IARC87a).

2,3,7,8-TCDD was not shown to cause the transformation of mouse embryo fibroblasts (IARC87a), but it was able to transform these cells following their prior treatment with N-methyl-N'-nitro-N-nitrosoguanidine (IARC87a). 2,3,7,8-TCDD was not capable of inducing unscheduled DNA synthesis in rat hepatocytes (IARC87a).

The Committee concludes from the foregoing that 2,3,7,8-TCDD has been examined thoroughly for genotoxic effects *in vitro* and that none of the many adequately performed *in vitro* tests, with the exception of the test using mouse lymphoma cells, justifies the conclusion that the substance has a genotoxic effect *in vitro*.

In vivo tests

2,3,7,8-TCDD scored negatively in the dominant lethal test in the rat (IARC87a). It caused neither structural chromosome aberrations in rat and mouse bone marrow nor sister chromatid exchanges in the bone marrow of the mouse (IARC87a, IPCS89).

In humans exposed to 2,3,7,8-TCDD during their work or as a result of accidents, no chromosome aberrations were observed in blood cells (IARC87a).

The Committee concludes that the data in question do not justify the assumption that 2,3,7,8-TCDD has a genotoxic effect *in vivo*.

Conclusion

The Committee believes that 2,3,7,8-TCDD has been studied extensively *in vitro* and *in vivo* for evidence of genotoxic effects, but that some relevant data are lacking (GR95). There are no data from *in vitro* tests for the detection of chromosome aberrations and *in vivo* tests involving material other than bone marrow. However, the Committee does not believe that the absence of such data is sufficiently serious to warrant the conclusion that the genotoxicity tests carried out preclude the reaching of conclusions.

The Committee attaches more weight to results obtained in *in vivo* tests than to those derived from *in vitro* experiments, in the event that the two sets of data are contradictory (GR95). Therefore, on the basis of the above results, it considers 2,3,7,8-TCDD not to be genotoxic, in spite of the positive results of one *in vitro* test. The Committee's conclusion is in agreement with that of the authors of the Criteria Document on Dioxins (RIVM93).

The Committee is unable to assess the remaining PCDDs and PCDFs in terms of genotoxic properties, since these have been studied too little or not at all in genotoxicity tests.

A.4 Carcinogenicity

A.4.1 Animal experiments

2,3,7,8-TCDD has been extensively studied for carcinogenic properties in the rat, mouse and hamster. For an overview of the relevant animal experiments, the Committee would refer to earlier evaluations (IARC87b, IPCS89, RIVM93). The results of these studies show that 2,3,7,8-TCDD is carcinogenic in all three types of animal studied, even in the hamster, an animal known to be relatively resistant to carcinogens. The results also show that this substance is carcinogenic in rats and mice

of both sexes and that it causes tumours in several different organs. Moreover, carcinogenic properties have been demonstrated in experiments where 2,3,7,8-TCDD was administered by a number of different routes. The Committee concludes from this evidence that 2,3,7,8-TCDD is carcinogenic in experimental animals.

The Committee considers the research by Kociba *et al*. (Koc78) and that done under the US National Toxicology Program (NTP) (NTP82a, NTP82b) to be appropriate in principle as the basis for risk assessment. Both studies spanned two years. In the former study the substance was administered in the feed, in the latter it was administered by means of a gastric tube or via the skin. The Committee endorses the conclusion of the authors of the Criteria Document on Dioxins (RIVM93) that the Kociba *et al.* study is the most suitable for risk assessment. It employed the administration route and duration most relevant for humans and produced a no-observed-effect level: the lowest of the experimental doses caused no significant increase in the incidence of any type of tumour. The lowest dose used in the NTP study did increase the tumour incidence, although this finding is consistent with that mentioned previously since that dose was higher than the lowest dose used by Kociba *et al.*

Kociba *et al*. administered 2,3,7,8-TCDD to male and female rats in their diet over a period of two years. The highest dose studied, 0.1 μg/kg of body weight, increased the incidence of tumour in the lungs, nose and tongue in both male and female rats. The incidence of liver tumours also rose in the exposed female rats. However, the incidence of several types of tumour which occur spontaneously in the strain of rats used and which increases with age, was reduced in the groups treated with 2,3,7,8-TCDD. The relevant tumours are those of the pituitary, pancreas and adrenal glands, cancer of the uterus and breast cancer. These reduced incidences could be the result of the anti-oestrogen effect of 2,3,7,8-TCDD. There were a number of indications, such as increased mortality, reduced weight gain and liver damage, that the dose used was toxic. These findings suggest that there are indications for a role for organ damage in the generation of the tumours, especially in the case of liver tumours.

The rats treated with the second highest dose, 10 ng per kg of body weight, demonstrated fewer signs of toxicity and no increased incidences of tumours. At the lowest dose, 1 ng per kg of body weight, there were no signs of toxicity or increased incidences of tumours.

Congeners other than 2,3,7,8-TCDD have not been studied in animal experiments, with the exception of a 1:2 mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin (NPT80a, NPT80b). This mixture increased the incidence of liver tumours in rats and mice following oral administration, but not after administration through the skin. The doses which raised the incidence of liver tumours also caused damage to the liver.

A.4.2 *Epidemiological data*

Epidemiological studies on the relationship between exposure to PCDDs and PCDFs and cancer are concerned almost exclusively with 2,3,7,8-TCDD. They involve exposure at work (Bon89, Col93, Fin91, Kog93, Man91) and exposure as a result of accidents where 2,3,7,8-TCDD was released (Ber93, Zob90).

Bond *et al*. studied the occurrence of cancer in employees who between 1937 and 1980 may have been exposed to 2,3,7,8-TCDD and PCDDs with six or more chlorine atoms (Bon89). This was a follow-up to an earlier study of an employee population of 2192 conducted for the period up to and including 1984. The researchers studied the group as a whole and carried out a separate analysis of the subgroup suffering from chloracne (323 employees), a skin condition which may occur following exposure to a large dose of 2,3,7,8-TCDD. They found no significant correlation between exposure and the total tumour incidence or the incidence of certain types of tumour on analysis of the group as a whole. In the chloracne subgroup, there was a greater incidence of tumours in connective tissue and other soft tissue.

Another retrospective study of employees was carried out among 1583 employees involved in the production of herbicides contaminated with 2,3,7,8-TCDD (Man91). It concerned the period from 1952 to 1984. Mortality caused by cancer among employees exposed to 2,3,7,8-TCDD in this period was compared to that in two control groups consisting of the general population and employees of the local gas company. The total cancer mortality was demonstrated to be significantly higher in those who had worked in the factory for more than 20 years and in those who had worked there since 1955. Mortality due to lung cancer and cancer of the haematopoietic system was also significantly higher.

The largest epidemiological study of the relationship between exposure to 2,3,7,8-TCDD and cancer is that of Fingerhut *et al*. (Fin91). This is a retrospective cohort study of 5172 employees of factories manufacturing products contaminated with 2,3,7,8-TCDD. The results showed that in the entire cohort no significantly increased incidence of the types of tumour associated with exposure to 2,3,7,8-TCDD - soft tissue sarcoma (STS), Hodgkin’s disease, non-Hodgkin’s lymphoma, cancer of the stomach, nasal cancer and liver cancer - had occurred. Only in the subcohorts with the highest exposures and the longest follow-up were there significantly more STSs and tumours of the respiratory tract. The researchers concluded that the observed effects were not great and could have been the result of exposure to 2,3,7,8-TCDD, but equally could be attributed to confounding variables such as smoking habits and simultaneous exposure to other carcinogens.

Collins *et al*. performed a separate analysis of the relationship between exposure to 2,3,7,8-TCDD and cancer in the group of 754 persons from the above study with the highest exposure levels (Col93). To this end they divided the group into two sections, one where chloracne was present (122 individuals) and a group where this was not the case (632 individuals). The analysis of the data for the first group - with the exception of the standardised mortality ratio (SMR) for STS (6.7; 95% confidence interval 1.4-19.4) - showed no significantly elevated SMRs. This group, however, was exposed not only to 2,3,7,8-TCDD, but also to 4-aminobiphenyl. A subgroup of persons with chloracne who were exposed to 2,3,7,8-TCDD but not 4-aminobiphenyl showed no increased SMRs. The researchers conclude that the increase in the incidence of STS is the result of confounding by simultaneous exposure to substances other than 2,3,7,8-TCDD.

One epidemiological study was carried out in a cohort of female employees put together on an international basis (Kog93) consisting of 701 women working in factories where 2,3,7,8-TCDD was released as an unwanted by-product of the manufacture of herbicides. In comparison with the population at large, the incidence of all types of cancer combined was higher among these women (SMR 2.22; 95% confidence interval 1.02-4.22). The researchers put forward two arguments to explain why they cannot relate the increased incidence unequivocally to exposure to 2,3,7,8-TCDD, the first being that the incidence proved to be highest in the group with the shortest follow-up time. The second is that the increase in the SMR was attributable to a great extent to melanomas in New Zealand women. However, animal experiments with 2,3,7,8-TCDD show no increased incidence of melanomas, and it is well known that the incidence of melanomas is higher in New Zealand women than in women from other countries. The researchers found that there was no increased incidence of breast cancer, the type of cancer which occurred most frequently in the cohort studied. They conclude that while their study does not indicate a relationship between exposure to 2,3,7,8-TCDD and cancer, it does not exclude the possibility of such a relationship.

Zober *et al.* (Zob90) reported the result of a 34-year follow-up study of 247 employees who were exposed for a short time to a high dose of 2,3,7,8-TCDD following an accident. The group was divided into three cohorts on the basis of exposure. SMRs for various types of cancer were calculated by comparison with the population at large. The total mortality figure of these employees did not differ from that of the general population. The SMR for all types of tumour combined was slightly but not significantly higher. A separate analysis of the small cohort of 114 employees with chloracne showed no significantly increased SMR for all malignant tumours combined; in the subgroup where exposure had taken place 20 years or more earlier, the SMR for all types of tumour combined was significantly higher. The authors

concluded that their study suggests that 2,3,7,8-TCDD is weakly carcinogenic. However, they point out that the study is small in size, too small to be able to demonstrate a weak effect, a doubling of the SMR in the whole group.

The accident in 1976 in a factory in the Italian town of Seveso where 2,3,7,8-TCDD was released, was so serious that the surrounding local population was exposed to this substance. Bertazzi *et al*. (Ber93) studied the incidence of cancer in the exposed population in the first ten years after the accident. For this purpose, the neighbourhood of the factory was divided into three zones according to the degree of exposure; residents of these zones were compared to a control group of 181 579 individuals from an area in the vicinity of Seveso which had not been exposed. The zone with the highest exposure showed no significantly increased risk of cancer; the number of persons exposed in this zone was small, however (724 persons). In the zone with the second highest exposure (4824 persons) the men showed a higher relative risk of contracting lympho-reticulosarcoma and the women of contracting liver cancer, multiple myeloma and myeloid leukaemia. In the zone with the lowest level of exposure (31 647 persons), STS and non-Hodgkin's lymphoma were slightly but significantly higher. The researchers also observed decreases in relative risks, namely in those of breast and cervical cancer in women. They conclude that the small effects observed may be attributable to the relatively short follow-up period.

The Committee concludes that the various studies do show a significant relationship between exposure to 2,3,7,8-TCDD and certain types of cancer, but that the picture is not consistent: many types of tumour are associated with exposure to 2,3,7,8-TCDD and the associations demonstrated are weak (low SMRs). The Committee believes it possible that there is a weak relationship between exposure to 2,3,7,8-TCDD and various types of cancer, but that the groups were too small and the follow-up period too short to establish such a relationship conclusively.

A.5 Research on the mechanism involved

2,3,7,8-TCDD was studied for tumour-promoting ability in the liver and skin and was found to be highly positive in both (Pit80, Pol82, Sil94). It was also studied for tumour-initiating ability, but demonstrated this to a very small degree or not at all (Gio77). The presence of tumour-promoting but not tumour-initiating properties is compatible with the lack of genotoxic properties.

In addition to 2,3,7,8-TCDD, its congeners 2,3,4,7,8-pentachlorodibenzofuran and 1,2,3,4,7,8-hexachlorodibenzofuran, were also studied for tumour-promoting ability (Heb90). They both demonstrated this ability, albeit to a lesser extent than 2,3,7,8-TCDD.

A.6 Risk evaluation

The Committee concludes from the data available that 2,3,7,8-TCDD is carcinogenic in experimental animals. It finds the data derived from epidemiological studies to be inconsistent and insufficient to warrant the conclusion that 2,3,7,8-TCDD is carcinogenic in humans. The Committee believes it possible that, following a longer follow-up of the cohorts referred to above, 2,3,7,8-TCDD will prove to be carcinogenic in humans. Nor do the available research results exclude the possibility that it causes cancer on exposure to higher doses. The inconsistency of the epidemiological findings means that an estimation of the potential risk of contracting cancer associated with exposure to 2,3,7,8-TCDD can be based only on the results of animal experiments.

The Committee has reached the conclusion that the carcinogenic properties of 2,3,7,8-TCDD are based on non-genotoxic activity. For this reason it advises the adoption of a recommended exposure limit based on the level at which no adverse effects were observed supplemented by a safety factor. In selecting the safety factor, account should be taken of observed differences in kinetics between humans and animals. The Committee considers the results of the study by Kociba *et al*. (Koc78) to be the most suitable for extrapolation on the basis of carcinogenic properties, provided that the results of further evaluation of this study (Goo92) are taken into account.

The Committee is unable to assess congeners other than 2,3,7,8-TCDD owing to the lack of sufficient data. For the risk assessment of other congeners, it proposes the use of the toxic equivalency factor (TEF), a measure of the relative toxicity of a congener expressed in relation to 2,3,7,8-TCDD, until sufficient data on these congeners become available.

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Annex **B**

The inquiry

The President of the Health Council of the Netherlands received the following letter, dated 11 January 1995, N° DGVgz/VVP/L 9552, from the Minister of Health, Welfare and Sport.

I hereby request the Health Council (also on behalf of my colleague from VROM) to produce an advisory report on the 'Criteria Document on Dioxins' drawn up by the RIVM.

The problem of dioxins has already received the attention of the Health Council on a previous occasion. The question of whether the contents in maternal milk, which are the result of the lifelong accumulation of these substances in the mother's fatty tissue, do not represent too great a risk to breast-fed infants was, at the time in question, a matter of central importance. The Council has, in two advisory reports dating from, respectively, 1986 and 1991, established a point of view on this matter. Also, in 1985 an advisory report was published on the subject of PCBs in maternal milk, a problem that cannot be considered separately from the dioxin problem, as became increasingly clear in the years immediately following the publication of that report.

The submitted 'Criteria Document on Dioxins' covers the literature up to 1992. It must be emphasised that, since that date, interesting new information has come to light, such as the draft evaluation document of USEPA and the results of the Dutch maternal milk project, as a result of which the Criteria Document is already somewhat out of date. I want to ask the Health Council to take this new data into account in making its assessment.

In the Criteria Document, the risks to man and ecosystems for the Dutch exposure situation are evaluated by means of a comparison with, respectively, the TDI and the MTRs for ecosystems and predators that are derived within the document. This means that, for the first time, a Criteria Document has

taken into account the possible consequences of secondary poisoning. Is the Health Council able to support the derived TDI and MTR levels and the conclusions of the evaluation?

In the evaluation, the starting-point for man was the TDI proposed by the WHO/EURO in 1990 of 10 pg of 2,3,7,8-TCDD/kg of body weight, and it was established that the TEF principle can be used to evaluate mixtures. What this comes down to in practice is a TDI of 10 pg TEQ/kg of body weight. This TDI is based on chronic toxicity — and carcinogenicity — experiments in laboratory animals, and takes into account differences in kinetics between laboratory animals and man. The basic assumption in this case is that the carcinogenic properties of 2,3,7,8-TCDD do not rest on a genotoxic working mechanism, and that an effect-threshold approach can be used as a starting-point. Does the Health Council share the opinion that 2,3,7,8-TCDD must be considered a non-genotoxic carcinogen?

Research results that became known after the Criteria Document was completed, such as the Dutch maternal milk study referred to above, and the USEPA evaluation, cause us to suspect developmental disturbances resulting from perinatal exposure are important for the risk evaluation of dioxins. What is the opinion of the Health Council on this subject? Is it possible, in the Council's opinion, to evaluate which is the more critical exposure in this respect: prenatal (in utero) exposure or postnatal exposure via the maternal milk? Can the Council indicate whether this fact has consequences for the setting of the exposure norm (TDI)?

In addition to dioxins, PCBs are also present everywhere in the environment. The result of the similarities in physical-chemical behaviour is that there is common exposure to both substance groups. A number of PCBs that, in terms of spatial structure, are comparable to dioxins, also display the same toxic effects as dioxins. This factor is currently taken into account in risk evaluations. For these PCBs, toxic equivalency factors have also been proposed (WHO, 1993). Is the Health Council of the opinion that a risk evaluation of dioxins and PCBs should be conducted in combination? If so, how can this be given expression in the setting of norms?

I would deem it a favour if the Health Council would publish its advisory report by, at the latest, the autumn of 1995.

Yours sincerely,
The Minister of Health, Welfare and Sport.
signed: E. Borst-Eilers

Annex **C**

The Committees

Committee on Risk Evaluation of Substances/Dioxins:

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Annex **D**

Procedure followed in the compilation of the literature

For the period from 1 January 1993 to 1 October 1995, a systematic search for articles was conducted in the scientific literature in the files of ‘Medline’ and ‘Toxline’, using the keyword ‘dioxins’. Reports of the Health Council of the Netherlands, the Rijksinstituut voor Volksgezondheid en Milieu (RIVM) and (draft) reports of the United States Environmental Protection Agency have also served as information sources. In addition, Committee members contributed both scientific articles and secondary literature. The literature list contains the publications that were selected by the Committee.

Annex **E**

Previous advisory reports issued by the Health Council of the Netherlands on Criteria Documents

The Health Council has previously issued the following advisory reports on criteria documents.

- Tri- and tetrachloroethane, N° 19, September 1985
- Acrylonitrile, N° 20, September 1985
- 1,2-Dichloroethane, N° 4, May 1986
- Vinyl chloride, N° 5, May 1986
- Ethylene oxide and styrene, N° 17, July 1986
- 'Fine particles', N° 40, December 1986
- Phenol, N° 9, April 1987
- Chloroform and tetrachloromethane, N° 19, September 1987
- Benzene, N° 20, September 1987
- Dichloromethane, N° 28, December 1987
- Propylene oxide, N° 29, December 1987
- Cadmium, N° 9, April 1988
- Hexachlorocyclohexanes, N° 10, April 1988
- Ozone, N° 23, July 1988
- Toluene, N° 29, November 1988
- Asbestos, N° 31, December 1988
- Copper, N° 9, April 1989
- Nitrates, N° 7, March 1990
- Fluorides, N° 10, November 1990
- PAH, N° 23, December 1990

- Chromium, N° 3, January 1991
- Chlorophenols, N° 9, April 1992
- Arsenic, N° 2, February 1993
- Radon, N° 3, April 1993
- Chlorobenzenes, N° 7, July 1993
- Particulate air pollution, N° 14, October 1995

Annex **F**

List of abbreviations

Below, the Committee gives the meaning of some of the concepts and abbreviations that are used frequently in the course of the report.

- *BCF*
  Bioconcentration factor; ratio between the concentration of a substance in the food of a predator and the concentration in the environment.
- *BMF*
  Biomagnification factor; ratio between the concentration of a substance in the predator and the concentration in the food.
- *BSAF*
  Biota sediment accumulation factor; ratio between the concentration of a substance in the organism and the concentration in the sediment.
- *Congeners*
  Compounds exhibiting a certain degree of similarity in chemical structure, e.g. chlorinated dibenzodioxins or brominated dibenzodioxins.
- *CYP1A1*
  Cytochrome P450 enzyme or messenger-RNA coding for it.
- *EROD*
  7-Ethoxyresorufine-O-de-ethylase; enzyme activity that is measured in order to determine the quantity of active P4501A1.
- *Isomers*
  Compounds with the same number and type of atoms (i.e. the same molecular formula), but with a different structure.

- K_{ow}
  Octanol/water partition coefficient; measure of the fat solubility of a substance.
- K_{oc}
  Sediment/water partition coefficient; normalised for organic carbon content.
- K_p
  K_{ow} x fraction of organic carbon (f_{oc}).
- *LOAEL*
  Lowest-observed-adverse-effect level.
- *mg, µg, ng, pg, fg*
  1 gram (g) = 10^3 milligram (mg) = 10^6 microgram (µg) = 10^9 nanogram (ng) = 10^{12} picogram (pg) = 10^{15} femtogram (fg).
- *NOAEL (NOEL)*
  No-observed-(adverse-)effect level.
- *PAH*
  Polycyclic aromatic hydrocarbons.
- *PCB*
  Polychlorobiphenyl.
- *PCDD*
  Polychlorodibenzo-*p*-dioxin.
- *PCDF*
  Polychlorodibenzofuran.
- *PCQ*
  Polychloroquarterphenyl.
- *P4501A1*
  Cytochrome P450 enzyme, involved in the biotransformation of foreign substances.
- *2,3,7,8-TCDD*
  2,3,7,8-tetrachlorodibenzo-*p*-dioxin.
- *TEF*
  Toxic equivalency factor.
- *TEQ*
  Toxic equivalent.