Dioxins
in the Environment

What are the health risks?

Synthesis and recommendations

This document presents the synthesis and recommendations of the group of experts assembled by the French Institute of Health and Medical Research (INSERM) in the framework of the procedure of Expertise Collective (expert advisory opinions). They responded to questions asked by the Directorate-General of Health and the Ministry of Land Use Planning and the Environment about the impact of dioxins on the environment and on health.

The Centre for Expertise Collective of INSERM (INSERM SC14) coordinated this expert advisory group, in collaboration with the Department for Economic and Social Partnership for the preparation of the file and the documentation department for the research bibliography (Department of Scientific Information and Communication).

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Foreword

"Dioxins" comprise two families: the polychlorodibenzo-*para*-dioxins (PCDDs) and the polychlorodibenzofurans (PCDFs). They are released into the environment during natural processes and during high-temperature heat processes related to industrial activity. Specifically, the major sources of dioxins today are the incineration of household waste and the steel and other metallurgical industries. The reduction of emissions over the past ten years has substantially decreased human exposure. Nonetheless, past industrial activity means that dioxins are still present in several compartments of the ecosystem (soils, sediments…) and continue to contaminate the food chain to some extent. Food (milk and dairy products, meat, fish) is the principal source of population exposure.

The Directorate-General of Health and Directorate-General of the Administration and of Development at the Ministry of Land Use Planning and the Environment sought information and advice from INSERM, through the Expertise Collective procedure, about the impact of dioxins on the environment and on human health. These two departments wanted, in particular, a critical expert assessment about: the measurement of dioxin concentrations in different media, the best way to estimate population dioxin burdens, the inventory of the emissions sources and the modalities of transfer in the different compartments, especially soil, and finally the models most appropriate for treating short-term overexposure.

To respond to this request, INSERM established a multidisciplinary group of experts that brought together scientists with expertise in epidemiology, environmental epidemiology, risk assessment, food, clinical and molecular toxicology, chemistry, biochemistry, oncology, endocrinology, immunology, and pharmacology. Three speakers who were not members of the group gave presentations related to different types of assays.

The group's scientific analysis was structured around the following questions:

- What is the physico-chemistry of dioxins ? How are they formed ?
- What are their reservoirs and sources ? What are their consequences to the environment? By what pathways is the food chain contaminated ?
- What assay methods exist ? By what metrics can the results be expressed ?
- How are dioxins distributed throughout various tissues? How does this differ between species ? What biomarkers are sensitive to and specific for dioxin exposure ?
- What biological and toxic effects are observed in different animal species ? What effects are observed in humans from exposure to high and to low doses? What are the consequences of mother-child transmission ?
- By what mechanisms do dioxins act ? How important is the mechanism involving the *aryl hydrocarbon* (Ah) receptor ? How can the variability of dioxin action from species to species and sometimes even within a single species be explained ?
- What toxicokinetic parameters must be considered in assessing the toxicity of dioxins for animals and for people ? What models can be used for a risk assessment of dioxin exposure ?

Querying general and specialized bibliographic databases allowed us to select more than 1 600 scientific articles. This documentary collection was also enriched by recent French and international documents, listed below.

During nine working sessions organised between September 1999 and June 2000, the experts presented a critical analysis and synthesis of the work published on various aspects of this

subject. The last three sessions were devoted to drafting the principal conclusions and recommendations.

DOCUMENTS

Agence française de sécurité sanitaire des aliments (AFSSA). Dioxine: données de contamination et d'exposition de la population française. Rapport rédigé dans le cadre du groupe de travail : "Contaminants et phytosanitaires" du Conseil supérieur d'hygiène publique de France, section "Alimentation et Nutrition". June 2000

Comité de la prévention et de la precaution. Rapport d'activité 1996-1998

Compilation of EU dioxin exposure and health data. Report produced for European commission DG environment. UK Department of the Environment, Transport and the Regions (DETR), October 1999

Dioxin and Furan Inventories, National and Regional Emissions of PCDD/PCDF. United Nation Environment Programme (UNEP). Prepared by UNEP Chemicals, Geneva, Switzerland, May 1999

Emissions de dioxines par les incinérateurs de déchets ménagers. Exploitation des mesures réalisées au titre de l'année 1997 à l'émission des installations de capacité supérieure à 6 t/h. Hervé Pernin, ADEME, October 1998

Etude sur les dioxines et les furanes dans le lait maternel en France. InVS/CAREPS, 2000

Exposure and Human Health Reassessment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and Related Compounds. US Environmental Protection Agency (*US EPA*), June 2000

Health Assessment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and Related Compounds. US Environmental Protection Agency (*US EPA*), August 1992, June 1993, January 1997

La dioxine et ses analogues, Rapport commun N°4, Académie des sciences, Comité des applications de l'Académie des sciences, Institut de France, September 1994

L'incinération des déchets et la santé publique : bilan des connaissances récentes et évaluation du risque. Collection Santé et Société, Édition de la Société française de santé publique, n°7, 1999

Monographs on the Evaluation of Carcinogenic Risks to Humans. Polychlorinated Dibenzo-*para*-Dioxins and Polychlorinated Dibenzofurans. IARC Lyon France 1997, **69**

Synopsis on Dioxins and PCBs. Compiled by Jouko Tuomisto, Terttu Vartainen and Jouni T. Tuomisto. Kuopio, Finland, 1999. Julkaisija – Publisher : Kansanterveyslaitos (KTL) Helsinki ; KTL Division of Environmental Health, Kuopio, Finland . Internet access: http://www.ktl.fi/dioxin

WHO European Centre for Environment and Health (WHO-ECEH). International Programme on Chemical Safety (IPCS). Assessment of the Health Risk of Dioxins; Re-evaluation of the Tolerable Daily Intake (TDI), 25-29 May 1998, Geneva, Switzerland. WHO 1999

Synthesis

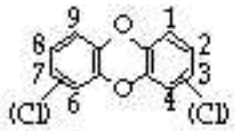
Dioxins comprise two main families, the polychlorodibenzo-*para*-dioxins (PCDDs) and the polychlorodibenzofurans (PCDFs), which, like the polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs), are members of the class of halogenated polycyclic aromatic hydrocarbons (HPAHs).

Dioxins are unwanted byproducts formed during many chemical processes involving chlorine, carbon, oxygen and a high temperature. In the developed countries, the two principal sources of dioxin emissions are both industrial -- the incineration of household waste and steel and other metal processing and production. Such emissions have diminished considerably over the past ten years in these countries. Nonetheless, dioxins are among the many pollutants to which populations are subjected at very low doses for their entire lives.

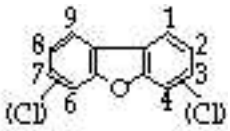
Dioxins are present in all the compartments of the ecosystem (air, soil, fresh and salt water sediments, animals). Because of their solubility in lipids and chemica stability, they are concentrated all along the food chain, and food has become the major exposure pathway for humans. Food can be monitored, especially if inspection of products of animal origin, dairy products in particular, is implemented. The problem lies in defining levels of tolerable exposure (standards, guidelines…) for preventive purposes. Establishing these requires an assessment of current knowledge about the toxic effects of dioxins in animals and in humans. Understanding its mechanism of action may also contribute to a better definition of the risk and facilitate its management.

Nomenclature and physico-chemical properties of PCDDs and PCDFs

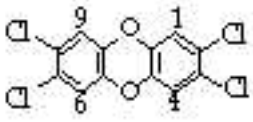
PCDDs and PCDFs are two families of distinct compounds, albeit very similar with respects to their molecular structure and their physico-chemical properties. They are oxygenated polycyclic aromatic compounds; the structure of PCDD has two oxygen atoms, while PCDF has only one. The numbered positions in aromatic rings can be substituted by hydrogen or chlorine atoms (no more than eight of the latter).



polychlorodibenzo-*para*-dioxin



dibenzo [b-d] furan



Formula for the dioxin at Seveso (2,3,7,8-TCDD)

Structural formulas of the basic compounds and the dioxin released at Seveso (2,3,7,8-TCDD)

There are 75 congeners of PCDD and 135 of PCDF; they differ by the position and number of chlorine atoms in the basic structure.

Number of PCDD and PCDF congeners

Number of chlorine atoms	Number of PCDD isomers*	Number of PCDF isomers
1	2	4
2	10	16
3	14	28
4	22	38
5	14	28
6	10	16
7	2	4
8	1	1
Number of congeners by family**	**75**	**135**

*isomers: molecules with the same atomic structure; **congeners: molecules with the same basic structure but with a different number of substituted atoms

The physico-chemical characteristics of PCDD and PCDF are closely linked to the degree of chlorination of their aromatic structures. They are only slightly volatile, only slightly soluble in water, but soluble in lipids. This lipophilic character enables them to cross cell membranes and accumulate in the fatty tissue of the organism.

PCDD and PCDF are stable up to 800° C and are not totally destroyed until 1 300° C. In the environment, photolysis is one of the rare pathways by which they decay. Photodechlorination appears to be the most important of these reactions. It involves the most chlorinated congeners in particular and may lead to the formation of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD) from octachlorodibenzo-*para*-dioxin (OCDD), the predominant compound in dioxin emissions. Biochemically, these compounds are highly stable, especially the most chlorinated of them. Nonetheless, several studies of their biodegradability have shown that some microorganisms (bacteria, yeast, fungi) can metabolize them.

Structure activity relationship

The cumulative and toxic properties of dioxins also depend closely on their chemical structure, that is, on the number and position of the chlorine atoms in the two benzene rings. Of the 210 congeners theoretically present in the environment after emissions from various point or diffuse sources, the 17 compounds substituted in positions 2,3,7,8 (that is, 7 PCDD congeners and 10 PCDF congeners) are the object of intense accumulation in living organisms, where they undergo a slow biological decay that varies according to the type of congener (more rapidly for PCDFs than for PCDDs).

In addition, these 17 congeners possess a steric conformation that promotes their binding to the intracellular Ah receptor, but their affinity for it does vary. It is highest for 2,3,7,8-TCDD and 10 to 10 000 times lower for the more chlorinated congeners (such as OCDD), which have a steric hindrance that limits binding to the receptor.

Among these 17 congeners, those that are likely to bind to the Ah receptor and which include at least 4 chlorine atoms in positions 2, 3, 7 and 8 are considered to be the most toxic. Toxicity diminishes as the number of chlorine atoms increases: the most toxic PCDD is 2,3,7,8-TCDD. At more than 5 chlorine atoms, toxicity falls sharply. The toxic potential of the 17 congeners

can be expressed relative to the most toxic compound by the concept of toxic equivalence (TEF, *toxic equivalent factor*). The development of this concept began in 1977 so that a toxicological value could be assigned to a mixture of chemically similar compounds with the same mechanism of action, that is, acting on the same receptor. Defined from *in vitro* results adjusted according to *in vivo* data, TEFs are reassessed frequently as knowledge progresses. This concept was first applied to PCDD/PCDFs and then extended to other members of the HPAH family. For PCDD/PCDFs, the reference congener is 2,3,7,8-TCDD, which has the strongest affinity for the intracellular Ah receptor.

Accordingly, the TEF is defined as follows:

$$\text{TEF} = \frac{\text{toxic potential of and individual compound}}{\text{toxic potential of 2, 3, 7, 8-TCDD}}$$

PCDDs	(N=7)	TEF	PCDFs	(N=10)	TEF
2,3,7,8-TCDD		1	2,3,7,8-TCDF		0,1
1,2,3,7,8-PeCDD		1	1,2,3,7,8-PeCDF		0,05
			2,3,4,7,8-PeCDF		0,5
1,2,3,4,7,8-HxCDD		0,1	1,2,3,4,7,8-HxCDF		0,1
1,2,3,7,8,9-HxCDD		0,1	1,2,3,7,8,9-HxCDF		0,1
1,2,3,6,7,8-HxCDD		0,1	1,2,3,6,7,8-HxCDF		0,1
			2,3,4,6,7,8-HxCDF		0,1
1,2,3,4,6,7,8-HpCDD		0,01	1,2,3,4,6,7,8-HpCDF		0,01
			1,2,3,4,7,8,9-HpCDF		0,01
OCDD		0,0001	OCDF		0,0001

Structure and TEF of the 17 PCDD and PCDF congeners with substitutions in positions 2,3,7,8 (• : chlorine atom)

Assay methods

Because dioxins are present in trace amounts, the complex analytic techniques used to identify them require very low thresholds of detection. These methods seek to assay the 17 congeners among the 210 possible congeners considered most toxic and bioaccumulative.

The analytic method of reference uses gas chromatography combined with high-resolution mass spectrometry (GC-MS). This method involves two successive stages: separation, based on the differential movement of hot gas compounds flowing through a capillary tube, and then detection, in which the molecular mass of the compounds is determined with high-resolution mass spectrometry. The method is very sensitive and very selective (detection limit in the order of 0.02 pg). The result of the analysis is expressed as a chromatographic profile of the congeners present; it can be converted into the total quantity of dioxins and into the weight of each congener.

The complexity, length, and cost of the analytic protocol are highest when the quantity of dioxins in the sample is lowest. Accordingly, the analytic protocols for PCDD/PCDF in products such as milk (contamination on the order of pg TEQ/g fat) are substantially different from those used to analyse such environmental samples as atmospheric emissions (contamination on the order of ng/m^3).

Progress in analytical chemistry separation techniques has improved the methods of extraction and purification, making them faster and less expensive. A protocol using microwave-assisted extraction, purification with high-performance liquid chromatography (HPLC), and an analytical method of gas chromatography combined with low-resolution mass spectrometry has recently been developed in France. This type of protocol should make it possible to satisfy requests for routine assays of environmental samples, without becoming incompatible with the requirements (of sensitivity and reproducibility) of the analytical method.

Methods that measure the biological activity of dioxins are complementary to chemical assays and provide toxicological results. These methods measure the quantity of dioxins as a function of Ah receptor activation, which leads to the expression of a reporter gene that can be measured (or whose products can be measured). The CALUX assay developed in the Netherlands uses a hepatoma cell line from a rat genetically modified by the introduction of a plasmid vector from the luciferase gene. Its transcription is controlled by a regulatory sequence of murine origin, named DRE (*dioxin responsive element*). Thus, in response to dioxin exposure, the cells synthesize luciferase, the enzymatic activity of which can be quantified by luminescent reactions. The quantity of light emitted is proportional to the activity of the Ah receptor. It is therefore closely linked to the type of dioxin congeners and to their proportion in a given mixture; its evaluation of toxicity is good. Other cell models based on this principle but measuring a different reporter gene can also be used (CAT system, *chloramphenicol acetyl transferase*).

Expression of assay results

The expression of the results of analytic assays varies according to the matrix used.

Expression of results according to the matrix

Matrix	Expression of results
Biological samples	ng TEQ/kg fresh weight or pg TEQ/g fat
Soil or sediment samples	ng TEQ or pg TEQ/g dry weight
Atmospheric emissions	ng TEQ/m^3

The concentration of each PCDD and PCDF congener in a mixture can be converted into an international toxic equivalent quantity (I-TEQ,), equivalent to the concentration of the PCDD or PCDF multiplied by its TEF.

Thus, the TEQ indicates the quantity of 2,3,7,8-TCDD necessary to produce the same toxic effect as that likely to be induced by the congener studied at the dose measured. For example, 30 ng of a congener with a TEF of 0.1 has the same effect as 3 ng of 2,3,7,8-TCDD. The TEF concept is based on the hypothesis that the doses and effects, both acute and chronic, are additive. It is therefore possible to compute the sum of the TEQ of each component of a mixture to estimate the overall toxicity.

$$\text{I-TEQ} = \sum(\text{TEF x concentration of PCDD or PCDF})$$

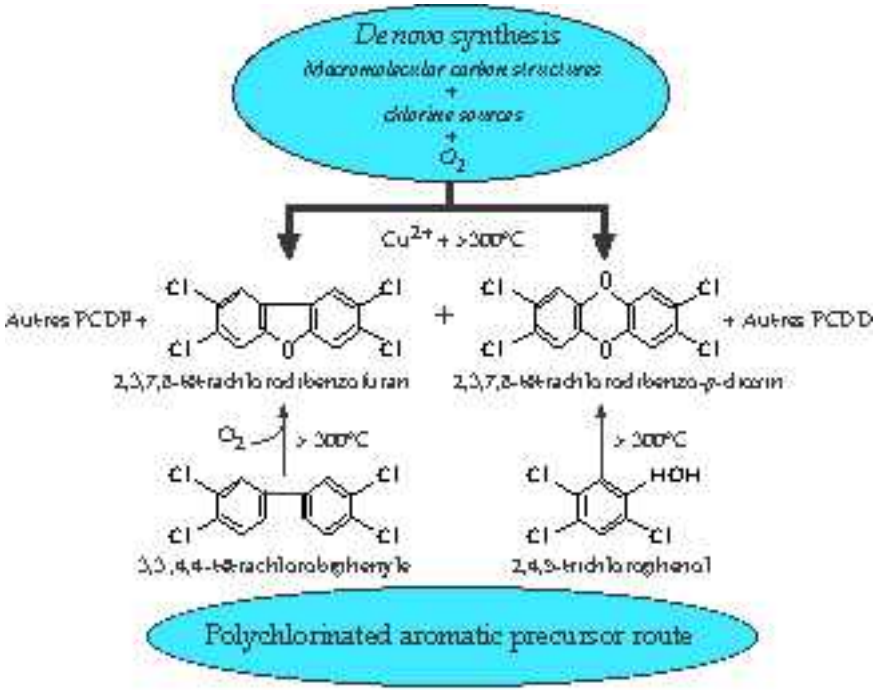
Expressing results in TEQ has limitations that make its use highly controversial, both for risk assessment and risk management. One limitation of the TEQ for risk management is that it does not provide information about the type of congeners involved in the contamination: therefore the source of the contamination cannot be identified. Moreover, the concept of additivity is also debatable when the TEQ is used for risk assessment, in view of the many examples of interaction -- antagonistic or synergistic -- in complex mixtures and of the probable existence of endogenous Ah receptor ligands. Finally, some of the toxic effects of PCDDs and PCDFs do not necessarily involve binding to the Ah receptor. Of all the compounds capable of binding to the Ah receptor, only the PCDD/PCDFs (NATO nomenclature) have been considered, together with, since 1997, the most dioxin-like PCB congeners (WHO nomenclature). The TEQ does not take into consideration the other "non-dioxin-like" PCBs, which are nonetheless the most abundant.

PCDD and PCDF formation

PCDDs and PCDFs are produced during most natural and industrial combustion processes, especially those involving high temperatures (incinerators, metal smelting…). They are also formed during the chemical synthesis of chlorinated aromatic derivatives and during natural biological processes and photochemical reactions.

To prevent PCDD and PCDF formation, it is essential to understand the processes by which they are synthesized. Many parameters influence this process and, despite numerous *in vitro* experiments on the topic, the reactive mechanisms involved remain little known today.

PCDDs and PCDFs are mainly produced in incineration ashes as the fumes cool. These ashes provide all of the essential elements for this synthesis -- residual carbon structures, chlorine and catalysts. This is called "*de novo*" synthesis. It is highly dependent on the presence of inorganic chlorine in the reactive medium. Hydrochloric acid and metal chlorinated derivatives such as copper chloride ($CuCl_2$) are the principal sources. Copper is also one of the most active catalysts of halogenation reactions in aromatic compounds. Oxygen, of course, is essential to the combustion of carbon structures and to the synthesis of PCDDs and PCDFs. *De novo* synthesis is today recognized as the principal production pathway for PCDD and PCDF.



PCDD and PCDF formation

It is nonetheless possible to synthesize PCDDs and PCDFs from organic molecules. This second pathway, referred to as the "precursor route", is taken by halogenated or hydroxylated aromatic compounds, such as chlorobenzene and chlorophenol, which play a role in the synthesis of herbicides (2,4,5-trichlorophenoxyacetic acid), bactericides (hexachlorophene...), and a wood preservative (pentachlorophenol or PCP). In this process, PCDDs and PCDFs are formed after the condensation and ring formation reaction of mono-ring precursors (Ullmann reactions). PCBs generate mainly furans through pyrolysis.

Principal dioxin reservoirs and emission sources

PCDD and PCDF emissions result essentially from human domestic and industrial activities. It is currently impossible to assess the importance of natural, chemical, biological and photochemical sources.

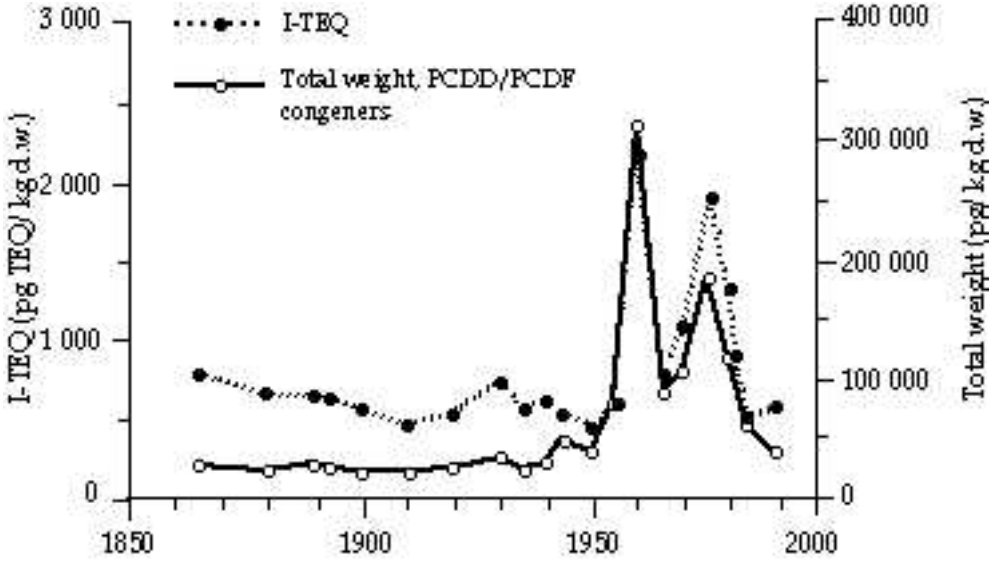
The history of dioxins is linked to the different sources that produce it, but also, at the same time, to the discovery, through scientific advances, of this family of pollutants. In the 1960s, PCDD and PCDF emissions were associated principally with industrial activities involving the synthesis of chlorinated derivatives (PCBs, PCPs, pesticides, etc.) and polyvinyl chlorides (PVCs)**.** The processes for manufacturing paper pulp (bleaching uses dichlorine) were also considered to be possibly important sources. PCDD and PCDF production resulting from these industrial activities has been substantially reduced after some products were banned and various technological processes modified. The recycling of non-ferrous metals, which involves the smelting of materials contaminated by chlorinated organic pollutants, also leads to PCDD and PCDF formation.

Among the accidental sources of PCDD and PCDF emissions, factories producing trichlorophenol and other chlorinated derivatives were important. The incident in the ICMESA factory at Seveso is the best known example. Changes in the manufacturing processes for these products should protect against this threat. Fires in warehouses, buildings and vehicles nonetheless remain a frequent emission source: the combustion conditions there are poor and the products that are burnt contain many elements that promote PCDD and PCDF synthesis (e.g., PVCs, fireproofing products).

Later, in the 1970s, it was demonstrated that dioxins form during combustion. Power plants (electric energy) as well as furnaces and boilers (for heating and industrial processes) emit PCDDs and PCDFs. Moreover, the more substitute fuels (waste reclamation and recycling) they use, the more dioxins they produce. Metallurgy and steel are the industries most concerned.

Over the past 20 years, municipal solid-waste and industrial waste incinerators have been the principal emitters of PCDDs and PCDFs, which are formed during the combustion process. Today, the development of effective technologies has limited the release of PCDDs and PCDFs by incinerator fumes. These improvements, together with a rationalisation of incinerator activities in Europe, have recently reduced this source of PCDD and PCDF production.

In England, the analysis of samples from an agricultural experiment station 40 km north of London made it possible to reconstruct the history of environmental contamination by PCDDs and PCDFs, from 1860 to 1993. Two contamination peaks emerged, during the 1960s (development of the chlorinated products industry) and the 1980s (entry into service of unprotected domestic incinerators). In Sweden, PCDD and PCDF assays of guillemot eggs (a sentinel bird species) similarly provided information about the history of environmental contamination by these compounds.



Time trends of PCDD and PCDF concentration from 1860 and 1993 in the herbaria of Rothamsted agricultural experiment station (England) (d.w.: dry weight) (from Kjeller et al., 1996).

European data show that the activities mainly responsible for dioxin emissions are incineration plants and the steel and metal industries, but that the contribution of each of these sectors varies from one country to another.

Distribution of dioxin emissions in France, Germany and the United Kingdom

	Distribution of emissions in 1995 (%)		
	France[1]	Germany[2]	United Kingdom[2]
Waste incineration plants*	46.00	10.0	87.0
Industrial combustion	2.63	5.5	5.8
Steel and metallurgy industries	51.20	83.0	7.0
Motor traffic	0.17	1.5	0.2

* : household and industrial waste; [1]: ADEME, 1996; [2]: *United Nations Environment Program*, 1999

In France, in a sample including 70 incineration plants and 80 steel and metallurgy sites, annual dioxin emissions diminished by more than 50% between 1997 and 1999. The paper, cement, and chemistry sectors each produce less than 1 g TEQ/year.

Time trend of dioxin emissions in the two principal sectors in France (data from Pollution and Risk Prevention Branch, Ministry of Land Use Planning and the Environment)

		Dioxin emissions (g TEQ/year)		
Activity Sector	Number of sites tested	1997	1998	1999
Household waste incineration plants*	70	500	300	200
Steel and metallurgy industries	80	350	300	120

***capacity greater than 6 tons per hour**

Some of the diffuse sources of PCDD and PCDF, such as exhaust emissions, motor oil, and home heating (wood, coal, gas) seem negligible. On the other hand, PCDD/PCDF reservoirs, such as PCP-treated wood, electric transformers containing PCBs, sewage sludge used as fertilizer, and contaminated soil and sediment, constitute potential sources of dioxins, whose real importance must be assessed. Recently, the detection of high dioxin concentrations in

clays and kaolins in geographic areas or soil layers not particularly accessible to recent pollution has focused research on natural dioxin sources. Studies of samples collected in the United States (Mississippi clay), Germany (kaolins), Australia and Asia (deep sediment) have confirmed contaminations from before 1900. The analytic profile shows a strong predominance of octachlorodibenzodioxin and suggests that dioxins may form naturally in sea sediment.

Contamination of environmental compartments

PCDD and PCDF contamination concern all compartments of the environment : air, soils and sediments, plants and animals.

The levels of atmospheric contamination depend on human industrial and domestic activities.

Dioxin contamination levels in the atmosphere of urban and rural areas in various European countries (*European Commission DG Environment*, 1999)

	Contamination level in air (fg/m^3)
Rural areas	
Great Britain (1991-1996)	1-24
Germany (1992)	25-70
Urban areas	
England (1991-1996)	0-810
Germany (1992)	70-350
Belgium (1993)	86-129
Netherlands (1991-1993)	4-99
Austria (1996)	26-314

Globally, the PCDD/PCDF concentration in soils depends on the (current or past) presence in the vicinity of potential sources, such as chemical or metallurgy plants or incinerators. Contamination occurs essentially by the deposition of atmospheric particles. The vertical migration of PCDDs and PCDFs in soils is minimal, and more than 90% of these compounds is found in the 10 cm topsoil. On the other hand, there is no indication that any appreciable loss occurs by either evaporation or decay over a period of several years (measured for 8 years) ; this points out the persistence of these compounds in superficial soil layers.

Spot European data are available: in Bavaria, where 90% of rural soil samples had measurements of less than 1 pg TEQ/g, higher contamination values were found in some forests. High concentrations were found near industrial pollution sources. In Germany, PCDD and PCDF levels in "industrial" soils were generally greater than 100 pg TEQ/g. In the Netherlands, values above 200 pg TEQ/g were recorded near municipal incinerators. In France, measurements taken in soils near an urban waste incinerator in operation for more than 10 years substantially exceeded 40 pg TEQ/g around the plume deposits.

PCDD/PCDF concentrations in the soils of various European countries (European Commission DG Environment, 1999)

	PCDD/PCDF (pg TEQ/g soil)
Rural areas	
Netherlands (1991)	2.2-16
Austria (1989; 1989-1993)	1.6-14 (pasture land); <1-64 (forests)
Germany (1992)	1-5
Belgium (1992)	2
France (1999)	0.02-1
Urban areas	
Germany (1992)	10-30
France (1999)	0.2-17
Industrial Areas	
France (1999)	20-60
Germany (1992)	50-150
Netherlands (1990-1991)	13-252 (municipal incinerators)

Contamination of sediment, like that of soils, depends on the pollution sources, the distance between the sampling area and the point sources, the circulation of water masses, and the dilution capacity of the fresh- or salt-water systems.

The data about plant contamination vary geographically as well as by the product sampled. Cabbage was selected as the sentinel species for atmospheric deposits in Germany. Values ranged from 0.7 pg TEQ/g in Hesse to 4.78 pg TEQ/g in Hamburg. In France, a study by the Directorate General of Competition, Consumption, and Fraud Prevention (DGCCRF) of plants collected near a household waste incinerator showed values ranging from 0.21 pg TEQ/g for cabbage to 1.10 pg TEQ/g for lettuce.

PCDD/PCDF concentration in sediments of various watercourses in Europe (*European Commission DG Environment*, 1999)

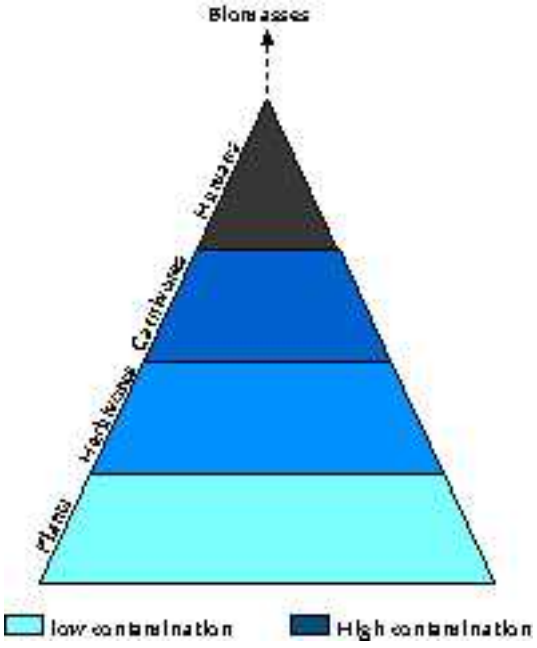
	Sampling Years	PCDD/PCDF (pg TEQ/g sediments)
German rivers	1994	1-20
Elbe Lower-Saxony	1994	1.17-19.2
Hamburg	1995	17.5-76.0
Rhine (North Rhineland/Westphalia)	1989-1996	16-103
	1995-1996	11-37
Port facilities (Hamburg)	1993	1 500
Rhine estuary (Netherlands)	1980-1990	8-21

Vegetables can be contaminated by various routes of exposure and penetration. Generally, the transfer of organic compounds from roots to leaves is negligible. Studies in growth chambers show that the principal source of the contamination of fruit and vegetable leaves is dioxin dispersion from the soil, even more than its absorption by the roots. In outdoor media, however, atmospheric deposits are the principal source of such contamination. Organic compounds in atmospheric deposits can thus penetrate into the plant leaves directly, crossing the cuticle or the stoma. We can use some German data to calculate a mean annual deposit rate per unit of surface, as a function of the atmospheric contamination levels. For both rural and urban areas, a mean factor of 200 can be calculated (for example, an air

concentration of 25 fg/m^3 corresponds to deposits of 5 pg/m^2 per year). For particulate emissions with high dioxin concentrations, this factor can be 10 times greater.

Transfer into the food chain

In every medium, food chains can be represented as trophic or food pyramids : the biomass at the diverse levels (plants-herbivores-carnivores) decreases substantially. This explains why the concentration in each of the nutritional levels of such pollutants as PCDD and PCDF, which are liposoluble, stable, and not very biodegradable, increases in inverse proportion to the reduction in the biomass. Moreover, each trophic level can be exposed to environmental sources that are added onto the food sources; for PCDD and PCDF, however, food exposure appears predominant, compared with other sources.



Concentration of bioaccumulative toxic substances by the food chains

Analyses of dioxin transfer towards animals in aquatic systems have examined fish, mollusks and crustaceans. These species accumulate more dioxins than terrestrial animals (e.g., cows, pigs, poultry) ; concentrations of several hundred pg TEQ/g fat were detected in fish.

Data for terrestrial animals concern mainly cow's milk. Pollution sources such as incinerators emit very little PCDD and PCDF in gas form ; these are principally adsorbed onto particles emitted at levels varying from less than 0.1 to more than 100 ng/m^3. PCDD and PCDF are deposited onto soil and plants, in particular onto grasses and weeds, as a function of atmospheric currents and precipitation. Transfer from soil to grasses and weeds seems very limited. Grazing cows are exposed mostly by ingesting contaminated grasses or hay ; the contamination levels of grasses and weeds in an exposed area vary from 1 to 50 pg TEQ/g dry weight. Consumption of soil or other fodder (corn, beets) results in considerably lower intake.

Gastrointestinal absorption of dioxins is generally substantial, with bioavailability ranging from 60 % to 90 % in animals and humans. Capacity for elimination is low and varies from one species to another. Lactation is a major route for excretion. This explains the low half-life of dioxins in dairy cows, for example. Distribution throughout the organism essentially depends on tissue lipid content ; PCDD and PCDF concentrate in adipose tissues, as in bovine meat. A recent survey in France of meat samples purchased in various retail outlets

revealed that all the samples had detectable dioxin levels ; these were, however, less than 1 pg TEQ/g fat.

Fat mobilization during lactation accounts for the high dioxin levels found in milk and milk products. In France, in areas where there is no local pollution source, the mean levels of PCDD and PCDF in milk are currently less than 1 pg TEQ/g fat. When dairy cattle graze near an industrial incinerator that emits PCDDs and PCDFs (metal or industrial waste recycling), the levels can reach 50 pg TEQ/g fat, depending on distance and winds, but also on zootechnical indicators (e.g., production level, number of lactations). In Austria, values as high as 69 pg TEQ/g fat have been reported in the milk of producers located near a copper smelter.

In France, milk and dairy products have been monitored since 1994 by the Directorate General of Food (DGAL), especially near incinerators and industrial areas. Recently, a memorandum from the Ministry of the Environment required incinerator unit managers not only to meet a fume emission standard of 0.1 ng/m^3 but also to conduct measurements of the dioxin content in the milk of local cows. Based on the tolerable daily intake (TDI), maximum residue limits (MRL) for PCDD and PCDF were determined for some major food categories, including milk and milk derivatives. In March 1996, the Expert Advisory Committee at the Council of Europe proposed values that were also adopted in France. The establishment of MRLs for other food, including beef, is underway.

Recommendations for dioxin levels in milk (CSHPF, High Council for Public Health, France 1998)

Guidelines	Observation
1 pg TEQ/g milk fats	Considered to be "background levels", objectives to be met
3 pg TEQ/g milk fats	Maximum recommended value; if this limit is exceeded, seek the causes and reduce them
5 pg TEQ/g milk fats	Maximum tolerated value; if this limit is exceeded, the milk cannot be sold

Experimental and field data make it possible to establish transfer coefficients between the various compartments (air, soil, grass, fat, milk). For example, a bioaccumulation factor can be calculated from values in pg TEQ/g milk fat compared with concentrations in pg TEQ/g dry weight, measured in pasture land grasses; according to the Austrian data, this factor is equal to 2. This rate concerns transfers from environmental compartments and not from animal feed.

After the transfer coefficients have been determined, models can be proposed to predict the incidence of given levels of PCDD and PCDF emissions by an industrial source. The kinetics of dairy cattle decontamination after elimination of the contamination source can also be predicted ; the reduction of dioxin levels in milk is relatively fast (several months) because of the rapid kinetics of lactating cows. It is also necessary to take into account the behavior of the different congeners. Thus 2,3,7,8-TCDD has the highest bioaccumulation factor of all the PCDD congeners. This means that different congeners have different chromatographic profiles in soil and grass than they do in milk.

PCDD and PCDF concentrations in human fat are ten times higher than in bovine fat. This body burden in humans explains the concentrations of 10 to 30 pg TEQ/g that have been observed in breast milk.

Human exposure assessment

It is generally agreed that more than 95% of the mean population exposure comes from food, in particular from the ingestion of animal fats (milk and dairy products, meat, fish). The

greatest portion of intake comes from bovine products (milk and milk products, meat and organ meats) ; poultry and pork are less important sources (except when their feed is contaminated) because they are raised inside buildings. Fresh and salt-water fish, shellfish, and other products are quite variable sources that can sometimes result in relatively high dioxin levels in populations that consume them in large quantities.

Daily dioxin intake from food: data from France (French Agency for Food Safety, 1999), Denmark (*European Commission DG Environment*, 1999) and the US (EPA, 2000)

	Contribution of different foods (pg/person/day)		
	France	Denmark	United States [1]
Milk and dairy products	25.8	46.0	12.0
Beef and pork	4.7	59.0	12.5
Fish	9.3	19.2	11.5
Poultry	1.2	5.9	3.8
Eggs	4.0	3.0	0.5
Crustaceans	0.8	-	-
Shellfish	7.1	-	1.3
Total	52.9	133.3	41.6

[1]: WHO nomenclature 1998

Diverse studies have shown that between 1980 and 1990 the total quantity of dioxins ingested by adults in food was approximately 150 to 300 pg TEQ/day, which corresponds to a median value of 2.3 pg TEQ/kg body weight/day and a maximum value of 4 pg TEQ/kg/d. These evaluations appear to be valid for most industrialized countries, but a clear trend shows that these levels have been falling for the past several years. In France, in 1999, the French Agency for Food Safety (AFSSA) estimated the median value of the quantity of dioxins ingested at 1.3 pg TEQ/kg/d ; the 95th percentile value was 2.6 pg TEQ/kg/d. These levels are close to those recommended by WHO (1 to 4 pg TEQ/kg/d). Nonetheless, WHO includes in its TEQ calculation those PCBs described as dioxin-like, which contribute on average 50 % of its value. Daily intake expressed in TEQ is therefore underestimated in France ; instead it ought to be assessed at around 2 pg TEQ/kg/d, according to the WHO nomenclature.

Direct dioxin burden can be assessed by quantifying the congeners present in the lipid fraction of various human tissues (blood, breast milk, abdominal and subcutaneous fat). This measurement was introduced at the beginning of the 1970s and initially applied, often retrospectively, to assess the exposure of populations of chemical industry workers. This approach later made it possible to determine the type and degree of exposure for workers exposed to industrial activities in which dioxins were impurities of the manufacturing or production process. The degree of contamination of the populations of accidentally exposed men, women and children (Seveso, Vietnam, Yusho, Yu-Cheng) has also been assessed by assaying these 17 primary congeners. More recently, this method has been applied to establish the mean dioxin burden of the general population in various countries across the globe.

The assay of dioxins in human tissues raises numerous practical difficulties. These assays are expensive. Moreover, a sufficient quantity of each sample must be obtained. Sampling subcutaneous or abdominal adipose tissues is an invasive procedure difficult to apply in epidemiologic studies. Analysis of breast milk, easily accessible and rich in fat, provides exposure information for only a small fraction of the population. Blood, because of its very low levels of circulating lipids (0.5 %), must be sampled in relatively large quantity (between 50 and 100 ml) and under adequate conditions (fasting and distant from any fat-rich meal).

Nonetheless, progress in these analytic procedures have already made it possible to assay dioxins in 10 to 15 ml of blood and even in less than 1 ml for relatively high degree of contamination. The assay also requires substantial skills in analytic chemistry. Moreover, the interpretation of the results of the assay of the 17 congeners in human tissue to assess human exposure must consider several parameters.

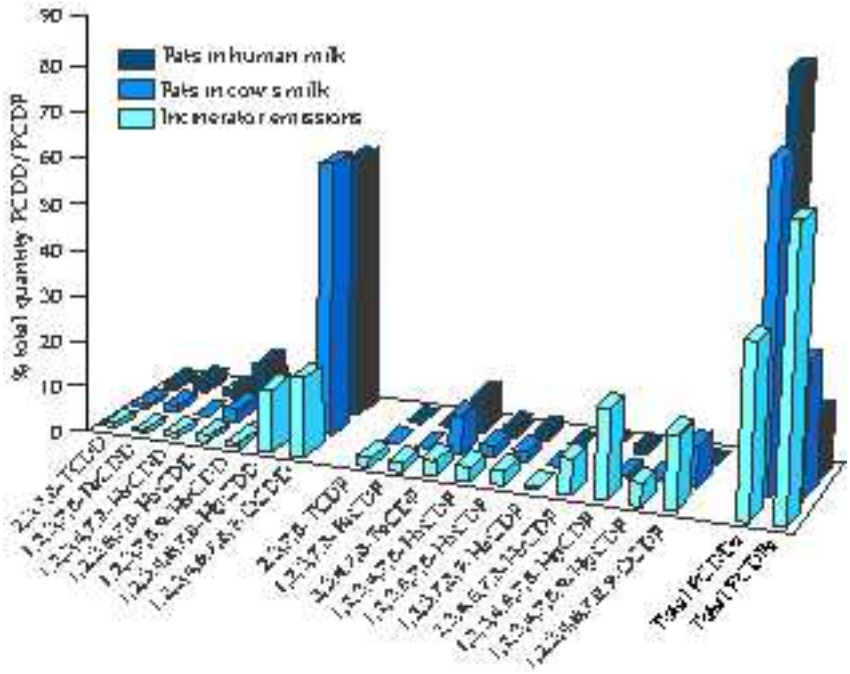
First, because dioxins accumulate, their measurement provides a good estimate of the body burden, that is, of the cumulative (throughout the subject's lifetime) internal dose, essentially from food, in the fatty tissues, before the sampling. In fact, for a constant exposure, the body burden stabilises at the end of 5 successive half-lives (the accumulation time being equivalent to the elimination time), that is, after more than 40 years for most congeners (mean half-life approximately 7 years). At this point, the body burden corresponds to the equivalent of 5 000 cumulative daily doses. It is thus clear that a transient exposure, such as the consumption of contaminated products for a brief time, must be quite substantial to modify the body burden. This modification can be assessed with pharmacokinetic models.

Because of the particularly long half-life of dioxins in humans, the assay of these substances in human tissue makes it possible to assess the history of exposure with one spot assay. This assessment method, however, cannot show any recent modification in the extent of food chain contamination or daily intake. For this reason, assays in the important vectors (cow's milk) are a useful complement to measurements in humans. Dioxin assays throughout the bovine industry provide a reasonably good estimate of recent environmental contamination, in view of the shorter dioxin life and elimination cycles in cow's milk.

Finally it is imperative that various factors affecting the dioxin concentration or body burden be considered in the interpretation of the values. Among the confounding factors that have been well studied, we note increasing age and increasing body mass, both of which increase body burden (except in small children), the number and duration of breast-feeding periods, which contribute to reducing it, and dietary habits, which are a major determinant (consumption of dairy products, beef, fish that are more or less contaminated depending on the geographic area).

In interpreting the biological significance of this type of assay, it is important to stress that no correlation has been established between body burden and the quantity of dioxins bound to the intracellular Ah receptor (or the effective dose responsible for biological effects). This association is all the more difficult to establish because diverse substances in the environment can act as agonists (Polycyclic Aromatic Hydrocarbons, such as those present in tobacco smoke) or antagonists (polyphenols) for this receptor. From this point of view, the biological assay methods (such as the CALUX technique) that make it possible to assess the degree of Ah receptor activation appear to be useful supplements to analytic dioxin assays and can be useful screening methods for identifying the subjects or populations most exposed to dioxins (and to other Ah receptor ligands).

Nonetheless, the analysis of the concentrations of each of the 17 congeners is an essential stage in the biological evaluation of dioxin exposure because it may contribute to the identification of contamination sources. The concentration of each congener integrates many factors : quantity present in the environment, bioaccumulation in the trophic chains, bioavailability after absorption, and metabolic transformation in humans (variable from one compound to another). The combined influence of these factors determines the relative proportion of each of the 17 congeners in the human tissue. For this reason, the congener profile in these tissues differs substantially from that observed in the environment and in various vectors (dairy products).



Representative PCDD and PCDF congener profiles in various matrices

The presence of different congeners cannot be quantified in the standard expression of the results of the 17-congener analysis as a toxic equivalent TEQ. The cumulative and toxic aspects of some congeners can sometimes be discordant : the best example comes from OCDD, which is a major component in the environment and the trophic chains ; on the other hand, its contribution to the direct biological activity of dioxin (TEQ) in the environment and in living organisms is quite minimal.

Population burdens

It is now well established that populations of chemical industry workers, like the populations of Seveso and of Yusho, were exposed to dioxins at levels more than 100 to 1 000 times higher than those of populations subjected essentially to contamination in their food. Moreover, the analysis of congener profiles within diverse populations demonstrates clearly that the type of congeners present in biological tissue depends on the circumstances of exposure (predominantly 2,3,7,8-TCDD for the population of Seveso, PCDF for those of Yusho and Yu-Cheng who had ingested rice oil contaminated by PCBs, and more PCDD than PCDF for the general population).

Blood concentrations recorded in populations exposed to dioxins

Population	Substances assayed (sampling date)	Concentration min-max (pgTEQ/g fat)
Highly exposed Populations		
Seveso (Area A) (1976)	2,3,7,8-TCDD (1976)	828-56 000 [1]
NIOSH (1951-1972)	2,3,7,8-TCDD (1987-1988)	ND-3 400 [2]
Ranch Hand - Vietnam (1962-1971)	2,3,7,8-TCDD (1987)	ND-618 [3]
General population		
Germany (1996)	PCDD, PCDF (1996)	6.1-41.5 [4]

ND: not detected; [1] from Mocarelli et al. 1991; [2] from Piacitelli et al. 1992; [3] from Roegner et al. 1991; [4] from Wittsiepe et al. 2000

The follow-up of diverse populations during the past two decades has made it clear that the mean burden in the general population of various countries has diminished (by nearly 50%). West Europeans are estimated to have a mean body burden of 100-200 ng TEQ. A study in Germany from 1991 through 1996 examined blood samples from boys and men aged 10 to 80 years. It found that the dioxin concentration fell during the study period by approximately 12 % per year ; it also confirmed the augmentation in dioxin concentrations with aging.

The population burden can also be assessed by levels in breast milk. The results from various European countries are fairly convergent, even though it is difficult to compare them : many involved only a small number of subjects, with samples that are generally pooled and collected in conditions that are not always described in detail. In France, a cross-sectional study assessed 244 samples of breast milk from around the country in 1998 and 1999 ; it found the means (arithmetic and geometric) and median all to be around 16 pg TEQ/g fat (standard deviation 5.15), similar to that in other European countries.

PCDD/PCDF levels in breast milk in Europe

Country	Year	Concentration (pg TEQ/g fat)[1] (min-max)
Germany	1993	16.5
Austria	1993	10.7-14.0
Belgium	1993	20.8-27.1
Denmark	1993	15.2
Spain	1993	19.4-25.5
Finland	1993	12.0-21.5
France*	1998-1999	16.4 (6.5-34.3)
Great Britain	1993-1994	17.9
Netherlands*	1992-1993	22.4 (10.0-35.9)

[1]: WHO data (1996), except for France (InVS/CAREPS, 2000); * : measurements taken from individual samples – in the other cases, the measurements were made from one or more breast milk pools

Acute toxicity

The toxicity of PCDDs and PCDFs has been demonstrated experimentally in many animal species, but most toxicology studies have been performed with 2,3,7,8-TCDD. The lethal dose

50 (LD 50) varies substantially not only by species and strain, but also sex, age and route of administration. Thus, the LD50 (µg/kg) for oral administration differs by a factor of 8 000 between guinea pigs, the most sensitive species, and Syrian hamsters. With intraperitoneal administration, there was a difference of a factor of 300 between Long Evans and Han Wistar rats.

Lethal dose 50 (LD50) after oral administration of 2,3,7,8-TCDD in various species

Species/strain (sex)	LD 50 (µg/kg)
Guinea pigs Hartley (M)	0.6-2.0
Chickens NR	< 25
Monkeys rhesus (F)	70
Rats Sherman, Spartan	
(M)	22
(F)	13-43
Rats Sprague-Dawley (M)	43
Rats Fischer Harlan (M)	340
Mice C57BL/6 (M)	181
Mice DBA2/2D4 (M)	2 570
Mice B6D2F1 (M)	296
Rabbits/New Zealand	115
Syrian hamsters (M and F)	1 157-5 051

M: male, F: female

Progressive weight loss, reduced food intake, thymus atrophy and gastrointestinal hemorrhage are consistent toxic effects. Other characteristic signs of toxicity are present in the liver, skin and endocrine glands. The diverse hepatic lesions include steatosis, giant hepatocytes, inflammation and necrosis. Skin damages are chloracne, hyperkeratinisation, involution of the sebaceous glands, and sebaceous cysts, in particular, in monkeys (rodents are not good models for such topics). Alterations in proliferation activity and in the state of epithelial cell differentiation have been shown in both cell cultures and *in vivo*.

Acute toxic response after exposure to 2,3,7,8-TCDD in various species

	Species				
Response	Rhesus monkeys	Guinea pigs	Rats	Mice	Syrian hamsters
Hyperplasia or metaplasia					
Gastric mucosa	++	0	0	0	0
Intestinal mucosa	+				++
Urinary tract	++	++	0	0	
Bile duct and/or gall bladder	++	0		++	0
Lung: alveolar area			++		
Skin	++	0	0	0	0
Hypoplasia, atrophy, or necrosis					
Thymus	+	+	+	+	+
Bone marrow	+	+		±	
Testes	+	+	+	+	+
Other lesions					
Hepatic lesions	+	±	+	++	±
Porphyria	0	0	+	++	0
Œdema	+	0	0	+	+
Hemorrhages	+	+	+	+	+

0: no lesion observed; +: lesion observed; ++: severe lesion observed; ±: moderate lesion observed

Biochemical observations in the liver include enzyme induction, in particular of the type 1A1 cytochromes P450 (CYP1A1), hyperlipidemia, vitamin A depletion, and porphyria.

Immunotoxicity in animals

The immunotoxic effects of 2,3,7,8-TCDD have been studied in many animal species, including mice, rats, guinea pigs, rabbits, and monkeys. It has so far been impossible to define an immunotoxicological profile valid in all these species. Hypoplasia or atrophy of the thymus is one of the most marked effects in rodents, but provides no clear meaning about the animal's immunological status. Mice are substantially more sensitive than rats.

Immunotoxic effects of 2,3,7,8-TCDD: LOEL (Lowest Observed Effects Level) (from IARC 1997)

Species	Protocol	LOEL	Effect
Rhesus monkeys	25 ng/kg for 4 years, oral	0.642 ng/kg/d	↘ Lymphocytes
Marmosets	0.3 ng/kg/w for 24 w	0.135 ng/kg/d	↘ Lymphocytes
Mice C57BL/6	1 ng/kg/w for 24 w, IP	1 ng/kg/w	Immunosuppression Poor regeneration of cytotoxic T lymphocytes
Mice B6C3F1	10 ng/kg 7 d after fertilization	10 ng/kg at d7	Augmentation of viral infection (in progeny)
Guinea pigs	8-200 ng/kg/w for 8 w	8 ng/kg/w	Immunosuppression
		200 ng/kg/w	Poor response to tetanus toxin

IP: intraperitoneal; w: week; d: day

The intensity of the immune depression -- both cell-mediated and humoral -- varies according to species. The depressive effect on humoral immunity is constant in mice. Non-specific defenses (phagocytosis) are not affected. Diminution of resistance to an experimental infection is often, but inconsistently, observed. No data support the hypothesis that 2,3,7,8-TCDD may increase sensitivity to induction of autoimmune diseases. The mechanisms involved in this immunotoxicity have not been elucidated.

One particular aspect involves the immunotoxic effects of dioxins after *in utero* exposure. Animal data demonstrate that sensitivity to dioxins is clearly greater during this period.

Effects on animal reproduction and development

The effects of 2,3,7,8-TCDD on reproduction and development have been studied in numerous animal models. The results in rodents and in other species show that sensitivity varies greatly from one species to another, within the same species and even between different strains.

Effects on gamete production and fertilization were clearly shown. 2,3,7,8-TCDD has a negative influence on the size of reproductive organs, the number of spermatozoa, and the quantity of ovules available by follicular maturation. Nonetheless, the diminution of fertility has been moderate. Fertility is especially reduced when females have been exposed prenatally to 2,3,7,8-TCDD, and even more so when the exposure took place during organogenesis. The hypothalamic-pituitary axis seems to be the principal seat of these

dysfunctions. Rodents diminution in fertility is also associated with their decreased sexual activity.

Maternal exposure seems to have little effect on the implantation stage. The Ah receptor is expressed by mice embryo from the 8-cell stage, and the only earlier effect observed in some rodents involves differentiation of the trophectoderm.

The documented teratogenic effects concern sexual differentiation, cleft palates, hydronephrosis, tooth organogenesis in rats and mice, the central nervous system (chickens), auditory damage (rats), and thymus alteration in all the animal models. The mechanism of action of 2,3,7,8-TCDD implies a problem in the sequential appearance of embryonic growth factors (such as TGFß - *transforming growth factor*), which are essential to successful organogenesis.

Studies of tooth organogenesis in rodents clearly show that 2,3,7,8-TCDD disrupts the expression of different growth factors involved in tooth development and that dental enamel is the most sensitive tissue. This effect has been reproduced in human fetal tissue cultures.

During organogenesis, sensitivity differs substantially between species and between strains of a single species. The sensitive rodent strains can present cleft palates at 2,3,7,8-TCDD concentrations of 1 pg/mg of palate tissue.

In a study of female rhesus monkeys exposed for 4 years to 2,3,7,8-TCDD in their food, endometriosis was observed 10 years after the end of the treatment. Its incidence was directly correlated with 2,3,7,8-TCDD exposure, and its severity was dose-dependent.

The diminution of tetra-iodothyronine (thyroxine) observed in rats exposed *in utero* to 2,3,7,8-TCDD was statistically significant only in females. Macaque monkeys subjected to low doses of 2,3,7,8-TCDD (close to environmental doses in humans) showed significant damage in learning tests involving spatial relations. Learning tests related to colour also revealed damage, but the difference was no longer significant.

Effects on Reproduction and Development : LOEL (Lowest Observed Effects Level)

Species	Dose, route, duration of administration	LOEL	Effect
Rhesus monkeys	5 and 25 g/kg/d mother's food, 4 years	0.642 ng/kg/d 0.126 ng/kg/d 0.126 ng/kg/d	Fetotoxicity Endometriosis (mother) ↘Object recognition
Sprague-Dawley rats	1-100 ng/kg to the mother, chronic 30 g/kg/d to the mother, 6-15th d of pregnancy	10 ng/kg/d	Fetotoxicity
Long-Evans rats	50, 200, 800 g/kg/d on the 15th d of pregnancy	200 ng/kg/d	Formation of vaginal threads
Holtzmann rats	64 g/kg/d to the mother on the 15th d of pregnancy	64 ng/kg/d	↘ Reproductive capacity in males

Carcinogenic effects in animals

The dioxin most often used in carcinogenesis studies is 2,3,7,8-TCDD. Seven long-term experimental studies of carcinogenesis were carried out in rodents, three in rats, three in mice and one in hamsters. In rats and mice, the liver is the principal target of cancer, but other sites (thyroid, lung, oral cavity) can also be affected. The mean incidence of tumors

(adenoma and carcinoma) is almost 50%, with susceptibility very dependent on sex. In rats, females are substantially more sensitive than males, while in mice, on the contrary, males are most affected. This difference in susceptibility according to sex is inverse to that generally observed in these species during standard hepatic carcinogenesis studies.

The *US Environmental Protection Agency* (*US EPA*) qualifies 2,3,7,8-TCDD as the "strongest known carcinogen" and as a "complete carcinogen" precisely because, in animals, very low doses administered over a long period induce cancers. A complete carcinogen is a substance that, applied alone to an animal for two years, significantly increases the number of tumors. Nonetheless, 2,3,7,8-TCDD is not mutagenic and, unlike genotoxic agents, does not directly induce DNA lesions. Based on the entire body of available experimental data, the International Agency for Research on Cancer (IARC) estimated in 1997 that there was "sufficient evidence" that the activity of 2,3,7,8-TCDD in animals is carcinogenic.

Hepatic carcinogenic activity of 2,3,7,8-TCDD

Species	Effective dose (µg/kg)	Duration of treatment (w)	Tumors (%)
Rats Sprague-Dawley	0.1 /d	104	40 (F)
Rats Osborne-Mendel	0.5 x 2/w	104	25 (F)
Rats Sprague-Dawley	1.75 x 2/w	30	0
Mice Swiss/H/Riop	0.7 /w 7.0 /w	52 52	47 30
Mice B6C3F1	M: 0.5 x 2/w F: 2.0 x 2/w	104 104	34 13
Mice C57Bl x C3H	5.0 /w	52	19 (F) and 66 (M)

M: male; F: female; w: week; d: day

Based on what is now known about the carcinogenic effects of 2,3,7,8-TCDD, three hypotheses can be proposed about its mechanism of action :

- the inhibition of apoptosis promotes the survival of precancerous cells relative to normal cells ;
- the triggering of oxidative processes, *via* the Ah receptor, and the massive induction of CYP1A, provoke oxidative DNA lesions ;
- the cytotoxic effects of 2,3,7,8-TCDD at low levels induce a discrete regenerative proliferation that promotes the fixation of DNA mutations induced by another mechanism.

Carcinogenic effects in humans

Numerous epidemiologic studies have assessed how dioxins affect the development of cancer in humans. The most informative epidemiologic studies are those that examined the population of Seveso, who was accidentally exposed to dioxins (TCDD) in 1976, and the workers exposed in plants manufacturing herbicide, chlorophenol, and chlorophenoxy, all contaminated by PCDD or PCDF. These prospective studies made considerable efforts to measure the populations' exposure to dioxins. The exposure levels were 100 to 1 000 times higher than those in the general population.

Highly-exposed population cohorts

Cohort	Characteristics
NIOSH	United States, 12 plants
IARC	International, 10 countries, 20 cohorts
German	Germany, 4 plants
BASF	Germany, 1 plant
Boehringer	Germany, 1 plant
Dutch	Netherlands, 2 plants
Seveso	Italy, population of Seveso
US Air Force Ranch Hand	United States, Vietnam veterans

Slight excess risks for all cancers considered together were found in all the occupational cohorts for which PCDD/PCDF exposure was correctly assessed. At 20 years after the first exposure, this excess risk was on the order of 40 %. Higher risks were consistently found for the workers with the greatest exposures. At Seveso, the overall risk of cancer did not increase during the early analyses but has tended to rise over the past five years. Some studies have found a higher risk for particular cancers (lymphoma, multiple myeloma, soft-tissue sarcoma, lung cancer, liver cancer), but on the whole the results have not been consistent : no particular cancer predominates in these exposed populations.

Cancer mortality in the occupational cohorts highly exposed to PCDD/PCDF

Source	Number exposed	Number of deaths	SMR (95% CI)
IARC international cohort *	13 831	394	1.2 (1.1-1.3)**
NIOSH Cohort	5 142	40	1.6 (1.2-1.8)
German Cohort	1 279	105	1.3 (1.0-1.5)
Dutch Cohort	549	51	1.5 (1.1-1.9)
BASF Cohort	113	18	1.9 (1.1-3.0)

***20 years after first exposure; **for the most highly-exposed groups in the cohort**

Several observations follow from the examination of the results of these studies on cancer risk. First, there are few precedents of carcinogens that affect the risk of cancer but do not predominantly influence a specific cancer. Second, the excess risks found in the occupational cohorts were statistically very significant ; the role of chance can be excluded. Despite this, these results must be evaluated prudently, in view of the smallness of the global risks. Possible biases due to confounding factors such as smoking or exposure to other occupational chemicals cannot be totally ruled out. Finally, the strongest evidence comes from studies of subjects with exposure levels 100 to 1 000 times higher than that of the general population. Extrapolation of these results to the general population would require assuming that the effects at low doses are similar to those at high doses. In the current state of knowledge, no cases of cancer have been clearly attributed to dioxin exposure in the general population.

Other toxic effects in Humans

It has repeatedly been shown that exposure to relatively high doses of dioxins causes dermatological effects (chloracne). Nonetheless, no direct relation has been observed between the exposure level and this manifestation.

Studies of occupational workers and of the accidentally exposed population of Seveso have shown a transient elevation in hepatic enzymes. An increase in GGT (gamma glutamyl transferase) levels was observed in the children of Seveso shortly after the accident but had disappeared five years later. This was also the case for D-glucaric acid in children and adults. Ten years after exposure, the transaminase (serum glutamic oxaloacetic or pyruvic transferases) serum levels were not elevated.

An increased risk of cardiovascular disease and a modification of blood lipid levels (increased total cholesterol and triglycerides) were observed in some studies of occupational workers, at Seveso and in the Ranch Hand study (Vietnam veterans exposed to Agent Orange, a defoliant mixture highly contaminated by dioxins). Nonetheless, these results are not entirely consistent. An increased risk of diabetes was found at Seveso and among the Ranch Hand cohort. Overall, the results indicate an increase in cardiovascular mortality for the most highly exposed groups**.**

Mortality from cardiovascular disease for highly exposed populations

Populations	SMR* (95% CI)
BASF cohort	0.6 (0.2-1.3)
IARC cohort	1.7 (1.2-2.3)
Dutch cohort	1.9 (0.9-3.6)
Boehringer cohort	1.4 (0.7-2.8)
Ranch Hand cohort	1.5 (1.0-2.2)
NIOSH cohort	1.8 (1.1-2.9)
Seveso cohort	1.6 (1.2-2.5)

SMR:** ***standardized mortality ratio

Other effects have been described, including changes in thyroid function and neurological or neuropsychological effects, but they are based on only a few observations.

The human data about immunotoxicity are relatively abundant, but contradictory. Neither the type of agent (dioxin or dioxin-like) nor the extent of the exposure involved in these studies is known precisely. The extremely variable modifications reported do not allow any conclusion to be drawn about the immunotoxic potential of dioxins ; in the current state of knowledge, a realistic evaluation of the immunotoxic risk for exposed individuals or populations does not seem possible.

Non-carcinogenic effects on populations highly exposed to dioxins

Effect	Epidemiologic evidence
Dermatological effects (chloracne)	Proven association
Gastrointestinal effects and liver enzymes	Temporary elevation of liver enzymes
Cardiovascular diseases and lipid concentration changes	Positive association at high dose in most studies but results not entirely consistent
Diabetes	Increased risks for Seveso and Ranch-Hand (morbidity)
Thyroid function	Several small differences (↗reported for T4, TSH, TBG, and T3)
Neurological and psychological effects	Several effects reported for Seveso and Ranch-Hand (polyneuropathies, coordination problems). Data not consistent. No association with depression
Respiratory system	Discordant data. Irritation and reduction of forced expiratory volume in some studies
Urinary system	No major dysfunction observed in kidneys or bladder
Immune system	Data not consistent

T4: tetraiodothyronine (thyroxine); TSH: *thyroid-stimulation hormone*; TBG: *thyroxin-binding globulin*; T3: triiodothyronine

Effects on reproduction and development in Humans

Most of the epidemiologic studies about reproductive and developmental effects concern paternal exposure. These include the follow-up of groups highly exposed to 2,3,7,8-TCDD and other PCDDs, such as the Seveso and Ranch Hand cohorts, as well as the occupational populations. The power of most of these studies was too low for them to be able to detect an increase in the risk of malformations at birth, and the results about the risk of spontaneous abortions are inconsistent. The studies about occupational exposure to dioxins and Agent Orange show a non-significant increase in cases of spina bifida and congenital heart disease. After the accident at Seveso, there was no clear evidence of an excess of major malformations, but there was a modification in the sex-ratio at birth for children born to couples in the high exposure categories, with a clear predominance of girls over boys. This phenomenon was most pronounced among the offspring of those aged less than 19 years at exposure. The results about modifications in reproduction-related hormone levels are contradictory**.** Several studies have shown impaired fertility after accidental or occupational exposure. This essentially involves alterations in spermatogenesis: oligo-, astheno-, and terato-spermia. This damage has not been confirmed by all the studies, however.

In studies involving environmental exposure, there is almost ineluctably co-contamination by PCBs. The data collected about the development of children exposed *in utero* to doses close to the upper limit of environmental PCB doses are fairly consistent. In the Michigan cohort, as in the European studies (Netherlands, Finland and Sweden), birth weight, height and head circumference were all lower among the children most highly exposed *in utero*. In the Finnish study, some children had alterations in the enamel formation of their premolars, which might be related to exposure during breast-feeding. Swedish fishermen were found to require a longer interval until their wives conceived, proportional to the amount of fish they

ate. No results have yet been reported about the effects on the female reproductive system, such as endometriosis or impaired fertility. We also note in these studies a significantly elevated incidence of abnormal laboratory tests (without any clinical manifestations, however) as well as of damage to the thyroid, immune, and coagulation systems.

Neurodevelopmental damage was studied during the 1970s in the United States (in North Carolina and the Great Lakes region). These studies found an inverse correlation between PCB levels in children and their scores on developmental, cognitive and neuromotor tests. Neuromotor delay was corrected during the first year of life, but the developmental delays subsisted and, at 11 years, arithmetic performances were still poor. The European series (Netherlands, Germany) found analogous results, but the follow-up did not continue past the age of 3 and a half, when the alterations were still present. Although a high concentration of dioxins and PCBs are transmitted by breast-feeding, it is the levels at birth that appear to be determinant for the effects on neuro-behavioral development.

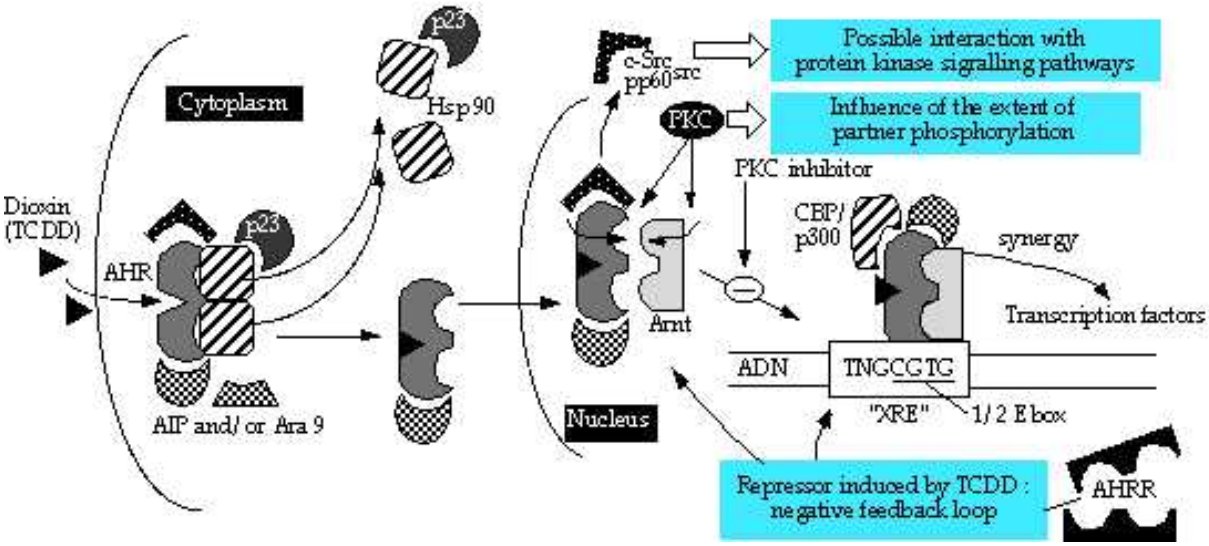
The epidemiologic data suggest therefore that, even during environmental exposure, the human fetus may be vulnerable to HPAHs. It should be stressed that the studies in question involve PCBs more than dioxins. Nonetheless, dioxin and PCB exposures are correlated.

Molecular cascade involving the Ah receptor

Recent years have witnessed considerable progress in the elucidation of the molecular mechanism responsible for the toxic manifestations of dioxin (with 2,3,7,8-TCDD considered the reference). The essential role of the Ah receptor in mediating some of the effects of 2,3,7,8-TCDD is now clear, thanks to recent work that led to the independent creation of three lines of transgenic Ah-receptor-deficient mice. When this gene is absent, so are the features usually described with dioxins (hepatotoxicity, cancers, malformations).

The structure of the Ah receptor, present in a quiescent state in the cytoplasm, is known in detail. It is similar to the nuclear receptors containing a PAS (Per-AhR-Sim) domain, which, because of their basic-helix/loop/helix (b-HLH) amino-terminal segment, can interact with the DNA, specifically with the conserved sequences (TNGCGTG) in the 5' region that regulates the genes transactivated by the receptors. The proteins with a PAS domain participate in the ancestral function of establishing circadian biological rhythms.

The activation of the Ah receptor by a ligand such as 2,3,7,8-TCDD leads to a cascade of events. The receptor, involved when inactive in a multiprotein complex, separates from its molecular chaperones (heat shock proteins Hsp90, p23, AIP (*AhR interacting protein*), ARA9 (*Ah receptor associated protein*)…) and can penetrate into the nucleus and dimerize with a partner protein named Arnt (*AHR nuclear translocator*). The phosphorylation of each of these partners modulates the capacity of the binary complex to interact with the DNA and to induce the transcription of target genes (cooperation with the transcriptional machinery and specific transcription factors). DRE (*dioxin responsive element*) sequences, recognized by the AhR-Arnt complex in the region regulating these target genes, are present in a considerable number of genes (cytochromes P450 1A, 1B…), all of which have not yet been identified. A feedback loop exists for these inductions : the Ah receptor induces the transcription of its own repressor, AhRR, a constitutive truncated form of the receptor, that competes with the native Ah receptor to recruit Arnt and form a transcriptionally inactive dimer.



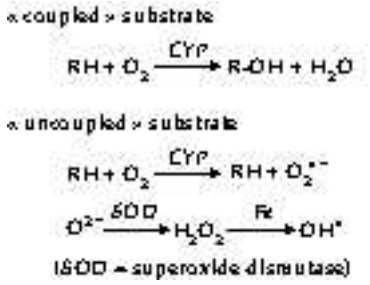
Molecular cascade involving the Ah (*aryl hydrocarbon*) receptor

Most 2,3,7,8-TCDD effects begin with the activation of the Ah receptor, which initiates a cascade of events involving different signalling pathways (protein kinases, phosphatases). These effects mostly follow the transcriptional signalling pathway of the Ah receptor, but the stages of its activation in the cytosol increase the availability of biologically active kinases. Activation of the Ah receptor can thus interfere with other cellular signalling pathways, which must be taken into consideration**.**

Comparative pharmacology studies have established affinity values of 2,3,7,8-TCDD for the Ah receptor that range from 1 to 10 nM for most species. These (relatively low) affinity differences cannot explain the variations in the levels of toxic effects observed between the different species. Taken together, the rodent lines with equivalent affinities for the Ah receptor have an LD50 that varies by a factor of 5 000. It appears that the affinity of 2,3,7,8-TCDD for the receptor is not the exclusive key to interpreting the inter-species disparities in sensitivity to this agent.

Consequences of the induction of cytochromes P450

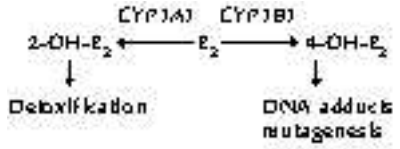
One of the best-studied and least-contested mechanisms of dioxin toxicity is the induction of oxidative stress. This stress can be measured in several ways : direct measurement of the reactive oxygen species (ROS), lipid peroxidation or formation of 8-hydroxyguanine. Dioxin may provoke oxidative stress by many different mechanisms. Induction of cytochromes P450 (CYP) is one possible route. The CYP are monooxygenases ; they are defined as "phase I" detoxifying enzymes, which metabolise xenobiotics, in particular polycyclic hydrocarbons. Phase I most often involves oxygenation of the xenobiotic, thus enabling the subsequent addition of a hydrophilic group by the phase II enzymes. During their catalytic cycle, monooxygenases produce reactive oxygen species (ROS) that are responsible for oxidative stress. In the presence of some substrates, known as "uncoupled", the oxygenation reaction is incomplete and produces oxygenated water (H_2O_2) or superoxide anion : the latter, transformed into H_2O_2 by superoxide dismutase, can yield a hydroxide radical ($OH^{\cdot}$) in the presence of iron (Fenton reaction). These oxygenated compounds are very reactive and can cause DNA lesions, alter proteins and modify lipids.



Production of Reactive Oxygen Species by Cytochromes P450 (CYP)

Dioxin induces the expression of three type 1 cytochromes P450 : CYP1A1, CYP1A2 and CYP1B1. CYP1A1 is the prototype of the gene inducible by dioxins. Dioxins also induce the expression of genes that code for phase II enzymes, which play a role in the detoxification of the metabolites generated by CYP1A1. The balance between phase I and phase II enzymes is therefore important in xenobiotic detoxification. Because CYPs metabolise many substances, both endogenous and exogenous, dioxin can modify this metabolism ; it is therefore necessary to consider the interactions between dioxin and xenobiotics such as benzo[a]pyrene, for example, in the case of smoking.

The cytochromes P450 induced by dioxin metabolise estradiol (E2). This induction participates in the anti-estrogenic effects of dioxin and, depending on the CYP affected, generates metabolites that may be non-toxic or genotoxic. CYP1A1 principally hydroxylates position 2 of E_2, thereby forming a nontoxic compound. CYP1B1 principally hydroxylates position 4, which yields a reactive metabolite capable of forming DNA adducts and therefore potentially genotoxic.



Simplified diagram of the metabolism of Estradiol (E_2) by cytochromes P450 (CYP)

One of the essential aspects of xenobiotic toxicity is the concept of individual genetically-based susceptibility. The difference in individual susceptibility may be due to a particular genetic profile, for example, in the genes involving xenobiotic metabolism and in regulatory genes. The performance of this type of study is just beginning, in an effort to better understand the effects of dioxin in humans. Individuals can be classed in 3 categories as to CYP1A1: highly, intermediately, or slightly inducible. Moreover, research has attempted to correlate the expression of xenobiotic metabolism genes or their polymorphisms with the onset of cancers in humans. The results for CYP1A1, still very preliminary, suggest an association between different variants of this gene and the risk of developing some types of lung cancer**.**

Mechanisms of action involving the Ah Receptor in endocrine diseases

The concept of endocrine disrupters is applied to molecules capable of imitating or blocking natural hormones, especially during the crucial stages of *in utero* life and development. Even before any direct agonist effect, dioxins disrupt the endocrine system by modifying steroidogenesis. The inhibiting effect exercised by dioxins on all the steroid hormones -- estrogens, progestins and androgens -- by modulating, in particular, their signalling pathways, might explain the pathogenic effects observed in the male genital tract and the

endometriosis in female monkeys and rats. Dioxins also seem to disrupt the effects of luteinizing hormone on ovulation.

Other mechanisms involving alterations in the endocrine function by dioxins may also be related to the toxic effects observed in animals and humans : disruption in thyroid hormone transport ; modification of cytokine expression and thymic involution in immunotoxic effects ; inhibition of the expression of growth factors (such as TGFß and EGF – *epidermal growth factor*) and of vitamin A in the onset of dermatologic features (chloracne) in humans ; inhibition of glucose transport, associated with the recent showing of diabetes in highly exposed populations ; interleukin disruption in some inflammatory diseases associated with dioxin exposure. Thus, for cardiovascular diseases such as atherosclerosis, reported in some cohorts, we might think that dioxins increase the expression of inflammatory factors (increased cytokines) that can provoke oxidative stress harmful to the endothelial cells of the vessels.

Our increasing knowledge of the multiple modes of action of dioxins suggests that these agents participate in the development of other diseases : osteoporosis (dioxins are anti-estrogens) and endometriosis (they are also potent anti-progestins). Dioxins may also be implicated in the development of neurodegenerative diseases, by inflammation and induction of specific genes.

Numerous research paths have opened ; the current goals are to identify the natural ligand of the Ah receptor and determine the pathophysiologic domain of this receptor according to the ligand. The discovery of new endocrine, metabolic, neurological or even viral target genes, inducible or repressible by dioxins, should help us to better understand the diseases associated with these agents.

Toxicokinetic models for risk assessment

Dioxin distribution in the body depends on the fat content of the tissues and their cytochrome P450 (CYP) concentration. At the concentrations usually found in humans, the effect of 2,3,7,8-TCDD sequestration by CYP is probably unimportant : the lipid fraction of the tissues is what determines its distribution. Fat mobilization during lactation diminishes the quantity of 2,3,7,8-TCDD in mothers, but transfers it to the child. The metabolism is the limiting factor in the (very slow) elimination of dioxins. The congeners with the fewest substitutions are the most rapidly eliminated. The metabolism process leads to the substitution of chlorine by hydroxyl groups and possibly to the formation of dichloro-catechol. The metabolites are eliminated in the bile.

Animal data indicate that the half-life of 2,3,7,8-TCDD in the organism is from 10 to 30 days in mice and rats and approximately 1 year in monkeys.

Toxicokinetic models have been developed for two primary purposes : quantitative analysis of the animal molecular toxicology data, in order to elucidate the mechanisms of action ; determination of human exposure and the half-life of 2,3,7,8-TCDD, either retrospectively, as in the Ranch Hand and Seveso epidemiologic cohorts, or prospectively, for the assessment of human health risks.

Toxicokinetic analysis of the human data indicates that the elimination half-life is approximately 8.5 years for the occupational cohorts and 15.5 years for the general population. This half-life varies greatly between individuals (for example, according to their body weight) and increases with age : some individuals have 2,3,7,8-TCDD elimination half-lives that go up to 30 years. Overall, humans accumulate much more 2,3,7,8-TCDD than animals do.

Physiological toxicokinetic models have been used to simulate and predict the effect, in animals and humans, of 2,3,7,8-TCDD on CYP induction, TGFß and EGF expression,

estradiol metabolism, the fate of vitamin A, and its action on thyroid hormones. The results on CYP1A1 and CYP1A2 induction and the diminution of EGF are compatible with a no-threshold model. Nonetheless, the carcinogenic effect of 2,3,7,8-TCDD in rat livers probably does not depend only on CYP action.

The aim of these models is partly to improve the transposition to humans of animal results, without introducing arbitrary safety factors. These models can be used for a detailed assessment of the risks of 2,3,7,8-TCDD, in particular, in cases of accidental contamination.

The toxicokinetic profile of dioxins partly explains the interspecies differences in toxicity and indicates that humans are more susceptible because of their longer lifetime and larger body burden. In view of the uncertainties about the importance of dioxin accumulation in the liver for some species, it is not unreasonable to base risk estimates on the body burden. The body burden in humans is highly dependent on the half-life of the particular agent considered, and recent studies show that this parameter varies greatly in the population. The risk assessment must therefore take into account the existence of populations sensitive to the effects of dioxins.

Assessment of risk for low-dose chronic exposures

The risk assessment procedure for dioxins proposed by the US National Academy of Sciences (1983) pays particular attention to the stage estimating dose-response relations. Deterministic effects, whose seriousness is dose-dependent, have a threshold of action ; they can be qualitatively distinguished from stochastic effects, whose frequency is dose-dependent and for which it is generally agreed that there is no such threshold (e.g., for genotoxic carcinogens). It can be decided whether a given effect appears to be deterministic or stochastic from available knowledge of its mechanisms, or empirically, from adjustments of the dose-response curves.

Quantitatively, we define for deterministic effects either a No Observable Effect Level (NOEL) (if there is one) or a Lowest Observable Effect Level (LOEL). In this case, it is not a risk that is determined but rather a tolerable daily intake : the above-mentioned doses are divided by a series of safety factors. For stochastic effects, the slope of the dose-response curve must be calculated for a unit of daily intake. It is then possible to assess the risk of a fixed dose, or the dose for a given risk.

Dioxins binding to the receptor is considered sufficient to induce expression of the target genes at the current exposure levels for the general population. The dose-relation response here is in principle linear. At this stage, we cannot really distinguish between the gene induction attributable to dioxins and that attributable to other exogenous or endogenous ligands.

Based on what we know now, we cannot definitively establish a causal link between the level of expression of these target genes and the risk of cancer. We must select hypotheses to justify the choices of a deterministic or a stochastic approach to assess this risk. WHO (1998) has selected the deterministic approach and concluded that at today's observed daily doses, the risk of cancer associated with dioxins is probably zero. A working group of the *US EPA* (1997) has proposed a series of models for cancers (linearized multistage and Weibull multistage models) for the animal data and two models, additive and multiplicative, for the epidemiologic data. The results are compatible with linearity and with a lifetime unit excess risk[1] on the order of 10^{-2} to 10^{-3} for an exposure of 1 pg TEQ/kg/d.

Based on the animal data, WHO (1998) retained the assumption that the systemic effects occurring at lower doses were expressed by alterations in sperm quality and immune responses, by congenital malformations in the offspring of rats exposed during gestation, by damage to neuro-behavioral development in young monkeys whose mothers were exposed during gestation, and by an increased risk of endometriosis in these female monkeys. From these data, considering the toxicokinetic differences between species, and applying a safety factor of 10, WHO proposes a tolerable daily intake for humans of 1 to 4 pg TEQ/kg/d.

[1] The "lifetime unit risk" is part of the calculation of the expected number of cancer cases per year, which equals: "lifetime unit risk (here 10^{-2} to 10^{-3}) x population size (in inhabitants)/mean lifetime (70 years)"

Effects most sensitive to 2,3,7,8-TCDD in animals

Organs/ Type effect		Species (sex)	Effective dose
Carcinogenicity	Liver Carcinoma	Rats (F)	100 ng/kg
Development	Neurotoxicity ↘Object recognition	Rhesus monkeys	0.126 ng/kg/d
	Reproduction ↘ Number of spermatozoa Formation of vaginal threads	Rats Rats	64 ng/kg/d 200 ng/kg/d
	Immunotoxicity ↗ Sensitivity to viral infection	Mice	10 ng/kg (7th d)
Immune system	Immunosuppression	Mice	1 ng/kg/w
Others	Endometriosis	Rhesus monkeys	0.126 ng/kg/d

The *US EPA* (1997) group, modeling those data in the animal studies that were appropriate for this purpose, concluded that the alterations in sperm quality, metabolism of thyroid hormones and cholesterol, as well as liver retinol levels, induction of hepatic enzymes CYP1A1 and CYP1A2, and thymus atrophy evidenced a linear dose-response relation. Changes in sexual behavior, teratogenesis, and immunotoxicity were considered to be effects with a threshold. It was not considered possible to reach a conclusion for the other effects.

The scientific knowledge available today does not justify choosing one or the other of these approaches. If we assume that the risk increases according to the unit risk function estimated by the *US EPA* above the tolerable daily intake (TDI) defined by the High Council of Public Hygiene of France (CSHPF) of 1 pg TEQ/kg/d, we can determine the excess risk of cancers associated with such a dose. Applying the recent census data (March 1999) for the French population yields an estimate of 1 462 (95% CI: 859-2 493) and 2 407 (95% CI: 1 289-4 470) annual cancer deaths, depending on the model chosen (additive or multiplicative, respectively). Cancers induced by dioxins will continue to develop after public exposure is lowered to the irreducible level defined by the natural production of these compounds.

Risk assessment in the case of short-term overexposure

Accidents on the scale of that in Seveso or very elevated occupational exposures are in principle no longer a real threat today, because of changes in the manufacturing processes for the products concerned. Accordingly, the chemistry of chlorophenols (source of the Seveso accident) has lost much of its importance.

PCBs, with a commercial formulation that includes a small quantity of PCDF, an unwanted by-product due to the manufacturing method, caused two very important accidents, in Japan in 1968 and in Taiwan in 1979. Both cases involved the involuntary enrichment by PCDF of PCBs, used as heat transport liquids in plants that manufactured rice oil. The leakage of this mixture of PCBs and PCDF into the food oil produced massive poisoning (at least 2 000 people exposed in each case). Since the mid-1980s, however, PCBs are not supposed to be sold.

Some large companies with a large stock of PCB-containing transformers have eliminated these transformers and replaced the PCBs by other products. There is in principle no further

danger of spot fires in operating transformers that could generate dioxins. On the other hand, the storage of discarded transformers could cause incidents, such as PCB leaks. Moreover, transformers that once contained PCBs may still be in service. The PCBs that they contained were replaced by other insulating fluids (mineral oil, in particular) that might be contaminated by PCB traces and therefore produce PCDF in any fire. In such accidental situations, it would be useful to sample all combustion products systematically, so that the quantity of any PCDF produced could be estimated.

There remain sources, probably very few, of overexposures that would be brief, but perhaps frequent. One group at risk is composed of scrap metal dealers, during the recycling of copper in transformers : because it is very often heated during the recovery process, it is likely to generate a relatively small quantity of PCDD and PCDF.

Because food is the major source of human contamination by dioxins, some populations may be more exposed than others, depending on their dietary patterns. When food is contaminated, those who consume the greatest amounts of it will therefore be most exposed. This observation has led the High Council of Public Hygiene of France (CSHPF), to consider guidelines (values not to be exceeded) for each category of food. The objective is to protect the population, including the largest consumers (95^{th} percentile) from ever exceeding the threshold recommended by WHO (4 pg TEQ/kg/d).

Recommendations

Population exposure to dioxins occurs primarily (95%) by the ingestion of food. Exposure by inhalation is negligible. Food is contaminated by the accumulation of dioxins present in the various environmental compartments, with particularly elevated levels in food from animal sources (milk and dairy products, meat, fish).

The environment has essentially been contaminated over the past five decades from two principal sources. The first is the chemical industry, which from the 1950s and into the 1970s manufactured numerous chlorinated products (polychlorinated biphenyls or PCBs, pentachlorophenol or PCP, 2,4-dichlorophenoxy acetic acid, or 2.4-D) that could contain dioxins (PCDD and PCDF) ; the dioxins were disseminated with the products into the environment. The second source involves the high-temperature processes related to industrial and domestic activities (steel production and other metallurgy, household waste incineration) that became the principal source of PCDD and PCDF emissions after chlorinated products were banned or limited. These emissions have been greatly reduced over the past decade, following changes in technological processes. In the developed countries, dioxin emissions into the environment have been cut to half or even a third of their previous levels. This decrease in emissions has reduced food contamination and diminished the population burdens (approximately 50% since 1990). It is nonetheless important to recall that, regardless of the actions taken against dioxins, there will always be a residual exposure due to the natural formation of PCDDs and PCDFs, principally by thermal (e.g., forest fires, volcanic activity) and possibly biological processes.

The expert advisory group, after analysis and synthesis of the literature and relevant data, proposes three lines of recommendation for action : pursue the reduction of food chain contamination by monitoring known emission sources and reservoirs, identifying dioxin sources and reservoirs not yet known, as well as inventorying the environmental contamination ; monitor the dioxin burden in general population and populations that are potentially more exposed or vulnerable ; establish a plan for intervention in the case of acute exposure.

The research recommendations involve several domains : the environment, with the proposal to reconstruct an environmental history to provide a scientific basis for French management options and to validate the transfer models between different environmental compartments ; public health, with the need to pursue epidemiologic studies of some effects that are still poorly understood ; molecular biology, to seek the mechanisms of dioxin's molecular actions, because of the many unknowns that remain ; finally, tools must be developed that will make it possible to improve the risk assessment.

Population exposure

CONTINUE TO REDUCE EXPOSURE IN HUMANS BY MONITORING THE FOOD CHAIN AND ACTING ON THE SOURCES AND RESERVOIRS OF DIOXINS

Food is the major pathway for PCDD and PCDF exposure in humans : contamination occurs by the ingestion of animal fat (milk and dairy products, meat, fish).

According to the recent data from AFSSA, the median value of the French adult population's overall exposure from food can be estimated at approximately 1.3 pg TEQ/kg/d, with a value at the 95th percentile of 2.6 pg TEQ/kg/d. These levels are close to those recommended

by WHO (1 to 4 pg TEQ/kg/d). Nonetheless, WHO includes in its TEQ calculation dioxin-like PCBs, which contribute on average 50 % of its value. Daily intake, expressed in TEQ, is therefore underestimated in France and should instead be assessed at around 2 pg TEQ/kg/d.

The expert advisory group recommends that plans for monitoring the food chain be drawn up and carried out. The government must turn its attention to the food vectors that contribute 80 % of exposure: milk and dairy products, fish, meat. These monitoring plans will make it possible to assess exposure through food, but also to follow up the effectiveness of measures for reducing the emissions sources in the environment and the contamination from various reservoirs. Given how dioxins are transferred in the food chain, the expert advisory group also recommends the surveillance of animal feed. This can only be imagined if carried out in cooperation with European authorities. The increase in upstream certification controls (certification of soils and of input into the chain) would avoid the multiplication of food product assays.

The experts also recommend that the surveillance of food products be performed by a chemical assay that can test for all 17 important congeners when necessary to identify a contamination source. The assays that are coming onto the market give results directly in TEQ and cannot establish the congener profile necessary for identifying the sources.

In several countries, the standard recommended for marketing milk is 5 pg TEQ/g fat. In France, the CSHPF has recommended a target value of 1 pg TEQ/g fat to reach a tolerable daily intake (TDI) of dioxins of 1 pg TEQ/kg/d for the population.

The increasingly specific knowledge of the distribution of dioxin levels in some foods (in particular, milk) makes it possible to determine mean values (0.6 pg TEQ/g fat in supermarket milk) and to assess the dioxin level above which a batch of food must be considered to be contaminated (2 pg TEQ/g fat for milk, for example).

For infant exposure, the recent French data about breast milk showed dioxin concentrations between 6 and 35 pg TEQ/g fat, with a mean of approximately 16 pg TEQ/g fat. This mean level entails a level of exposure to the baby of 70 pg TEQ/kg/d, during the first three months of life, well above the TDI. Nonetheless, this is calculated for a lifetime exposure. There is not now any justification for calling breast-feeding into question, since the possible risks of exposure (for example, neuro-developmental effects) that have been observed in some epidemiologic studies were seen in children fed with milk with a dioxin content between 30 and 60 pg TEQ/g fat, that is, higher than the levels observed in France. The development of breastfed children remains better than those of children fed with formula, as does their immune status.

To reduce food contamination in France, measures to identify emission sources and reservoirs of dioxin in the environment must be considered. Research on polluted soils should make it possible to exclude some contaminated areas from milk production. PCDDs and PCDFs must be assayed in sampling campaigns for soils and sediments, performed by the various relevant bodies. Assay results must be expressed in a manner appropriate to the objective sought : the results of analyses aimed at screening sources of contamination must be expressed as congener profiles while data intended to assess health risks must be expressed in TEQ.

The reduction of food contamination also involves surveillance of known sources of dioxin emissions. These sources result from industrial and domestic activities (steel production and other metallurgy, household waste incineration). An assessment has been made of the risks in France associated with incinerator emissions into the atmosphere and their consequences for food contamination. According to a theoretical calculation from the Caltox model, this exposure route (by ingestion) represented in 1997 approximately 30% of the TDI recommended by WHO (1 g/kg/d). If incineration emissions were brought up to the standard of 0.1 ng/m^3, it would become almost nonexistent (0.3% of the TDI). The expert advisory group recommends the rigorous application of the limits defined for emissions

from household and industrial waste incinerators for new and old systems, in accordance with the European guidelines.

The expert group believes that a plan for monitoring environmental contamination by PCDDs and PCDFs should make it possible to identify the contribution of reservoirs and diffuse dioxin sources : persistence or storage of PCB-containing transformers (because of possible leaks of the dioxin-loaded PCBs), PCP-treated wood, sludge from sewage retreatment, compost, and fertilizer, waste landfills, home heating or accidental diffuse sources (forest fires, fires of PCB-containing transformers)

ESTIMATE THE LEVELS OF THE DIOXIN BURDEN IN THE FRENCH POPULATION

The follow-up of diverse populations over the past two decades has clearly shown a substantial diminution (of nearly 50%) in the mean dioxin burden in the general population of Europe. In France, data measuring biological exposure to dioxins have recently been obtained in breast milk samples.

To learn the levels of this burden in the French population generally and in the most exposed populations in particular, the expert advisory group recommends as a first step the performance of a pilot study involving assays of blood samples from a sample of men and women aged from 18 to 80 years. In addition to data about the levels of dioxin burden and their distribution in the population, this pilot study should provide information to assess the interest and feasibility of a large-scale study. Such a study is justified by the need to validate transfer models (correlation between daily intake and body burden) and to evaluate the effectiveness of the decisions made in managing dioxin pollution, for example, by measuring the reduction in exposure (by monitoring the populations tested). Moreover, the expert advisory group recommends that a food questionnaire be used to assess the burden of some populations potentially exposed to greater contamination because of their particular dietary habits (higher consumption of fish and seafood, for example). In these groups exposed above the 95th percentile, the individual consequences of exposure may not be insignificant : it is important to study the actions likely to decrease their burden towards the mean value.

DEVELOP A PLAN FOR RISK ASSESSMENT IN THE EVENT OF ACUTE EXPOSURE

Accidents on the scale of that in Seveso are no longer a real threat today, because of changes in industrial practices. Short-term overexposure to dioxins can still occur, from both fortuitous events (fires in transformers or buildings, leaks of hydraulic or heat exchange fluids, leaks at sites where old transformers are stored or metals and electric cable are recovered, transport of household waste incineration emission purification residues (REFIOM) from incinerators…) and fraudulent practices.

The expert group recommends that all measures necessary be taken to prevent short-term overexposure ; these measures essentially consist of inventorying all potential sources (for example, PCB stocks) and progressively destroying or securing them.

Different European countries have selected three different criteria to assess food contamination : the dioxin levels in the product relative to usual levels (in Belgium) ; levels exceeding the tolerable daily dose (TDD) (in France) ; a significant increase in the body burden (in the Netherlands). Depending on the criterion selected, one might define a cut-off point for withdrawal from the market or exposure periods during which the continued sale would not significantly modify the body burden. For example, Dutch authorities estimate this period at thirty thousand days for eggs containing 60 pg TEQ/g fat and at 275 days in the case of equivalent contamination of bovine meat or dairy products. In France, the objective is to protect the entire population, including those who consume large amounts of each class of food (95th percentile) from exceeding the threshold of 4 pg TEQ/kg/day recommended by WHO. For this risk management option chosen, France shall soon define

the levels of PCDD and PCDF contamination that must be considered as cut-off points for banning the sale of food, other than dairy products, that contribute significantly to exposure.

If some foods are substantially contaminated, the harm will be both more limited and more containable to the extent that monitoring operations made possible both early detection and traceability, from which the extent of the damage can be estimated. A plan of withdrawal from the market may be established, in particular for foods containing more than 2% fat.

The expert advisory group recommends that the measures taken be proportional to the extent of the exposure (alimentary or environmental) : inform populations and health-care personnel ; determine the number of subjects exposed and contaminated and institute follow-up, in particular for the pregnant women and nursing mothers who were contaminated ; use toxicokinetic models to determine the duration of the follow-up necessary for the return to normal of exposed, contaminated subjects, and assess the impact of such an exposure on the lifetime body burden.

Research paths

DEVELOP RESEARCH THAT WILL IMPROVE THE IDENTIFICATION OF SOURCES

Until 1970, PCDDs and PCDFs, which are impurities produced during the chemical synthesis of chlorinated products, contributed to the contamination of the environment. Following the prohibition or restriction of some chlorinated compounds, studies in Sweden (of guillemot eggs) and England (of grasses) showed a diminution of environmental contamination by PCDDs and PCDFs. At the beginning of the 1980s, the construction and operation of many household waste incinerators seem to have caused these levels to rise again. Control of the technological processes of incineration and of metal reprocessing has since led to a renewed decrease in environmental contamination.

Reliance on the European data alone does not appear sufficient to rationalise the options for management of this risk in France. The expert advisory group recommends that, in addition to a soil inventory, a sample library should be constructed from sediments and/or herbaria, for both retrospective and prospective examination. This analysis would make it possible to estimate the contribution of different sources over time and to assess the current situation in different environmental compartments, in particular, soils and sediments. The expert advisory group suggests that, in this regard, all of the bodies with access to historic samples be identified and asked to participate in a coordination group. Meeting these objectives could also be facilitated by the establishment of a soil monitoring agency (or observatory).

STUDY THE TRANSFER AND THE FATE OF DIOXINS IN THE ENVIRONMENT

Effective risk management requires models of the transfer of dioxins from the soil →plants, from atmospheric deposits →soil, from ground water supplies →soil. Once contaminated soils and fields are identified and measured, a mean transfer rate can be calculated from the TEQ values of milk, in relation to the TEQ concentrations found in the pasture land grasses. This rate concerns transfers from environmental compartments and not from animal feed.

The expert advisory group therefore recommends that transfer models be developed and validated, through the collaboration of the various organisms concerned (French Institute for Agronomy Research (INRA), The French National Center for Scientific Research (CNRS), Bureau for Geology and Mining Research (BRGM), etc.).

Several works have examined the potential of microorganisms to biodegrade dioxins and thus regenerate the soils ; these bacteria, yeast, fungi, however, do not always appear to have a positive effect. This area should be explored and possibly exploited.

DEVELOP NEW ASSAY METHODS

The analytic method of reference for assays of PCDD and PCDF uses gas chromatography combined with high-resolution mass spectrometry (GC-MS). This is a very sensitive and selective method. The result, expressed as a chromatographic profile of the different congeners, can be converted into the total quantity and the weight of each congener present. The complexity and cost of the assay (approximately 5 000 F) tend to limit the number of analyses performed. Moreover, the great variability in the results between different laboratories complicates their interpretation.

Recently, progress in analytic chemistry separation techniques has improved the methods of extraction and purification and made them less expensive. Moreover, combining chromatography with low-resolution spectrometry, which is easier to use, should make it easier to perform assay series (for example, on environmental samples). The expert advisory group suggests a reinforcement in the development and coordination of research on analytic assay techniques.

Biological assay methods, based upon the estimate of dioxins bound to the Ah receptor, should expand in the years to come. These methods, less expensive and requiring smaller blood samples for assays of humans, will be more appropriate for public health monitoring. The expert advisory group recommends that the protocols developed in different laboratories be taken into consideration for defining and validating a standard technique. The possibility of performing such assays easily on blood samples would facilitate epidemiologic surveys.

DEVELOP TOXICOKINETIC STUDIES TO VALIDATE THE TRANSFER MODELS

These models involve the transfer of dioxins from the environment to humans. There are also physiological models that describe in a detailed manner the absorption, distribution, metabolism and excretion of dioxins. Finally, toxicodynamic models have been developped for studying the dose-response relation on which the risk assessment is based.

The expert advisory group recommends that these toxicokinetic and toxicodynamic (dose-response) studies be developed further in order to establish the limits of each of these different models. It underlines the need to reinforce skills and competence in France in the domain of modeling and risk assessment.

DEVELOP EPIDEMIOLOGIC STUDIES

Several occupational cohort studies have shown a slight excess risk for all cancers considered together (relative risk of 1.4). Some epidemiologic studies have shown an increased risk of cardiovascular diseases or diabetes, but they are not all consistent. The expert advisory group recommends encouraging the follow-up of existing cohorts for these effects.

In epidemiologic studies, PCDD and PCDF exposure is often associated with PCB exposure, which does not facilitate interpretation. Particular attention must be paid to new-borns and infants, because of transplacental exposure at a critical period of organogenesis and also because of the high exposure in children being breastfed by their mothers. This concern is supported, on the one hand, by all of the animal data, which show a greater sensitivity to dioxin effects (immunotoxicity, reproductive system effects, neuro-behavioral or thyroid effects) during *in utero* exposure, and, on the other hand, by preliminary results in babies that seem to indicate that *in utero* exposure affects some of these parameters. The expert advisory group also suggests that knowledge of this population of new-borns and babies be better developed. The studies that should be sponsored should integrate a better assessment of the exposure levels of dioxins and other pollutants. In view of the multiplicity of the effects of dioxins, it is also important to include in these studies different markers of clinical or biological effects (TSH, tooth enamel).

BETTER UNDERSTAND THE MOLECULAR MECHANISMS OF DIOXIN'S ACTIONS

The essential character of the Ah receptor in mediating the toxicity of 2,3,7,8-TCDD has been demonstrated : in the absence of the Ah-receptor gene in mice, the features usually described (hepatic, carcinogenic, teratogenic) are not observed. The activation of the Ah receptor initiates a cascade of events involving numerous signalling pathways. The experts recommend that the relations of the signalling pathways connected to those of the Ah receptor be studied and that other possible ligands, endogenous or exogenous, be sought. For some genes, much remains to explore about the final stage in the activation of their expression. The expert group also recommends studying the repertory of target genes and their function(s), according to species.

We do not currently understand why strains of rats whose Ah receptors have a comparable affinity for 2,3,7,8-TCDD have such different sensitivity to its effects. It appears that the receptor affinity for 2,3,7,8-TCDD is not the only key to interpreting the disparities in the sensitivity between species. Their inhibitory effects on the expression of growth factors and on cytokine disruption mean that dioxins can contribute to a large range of diseases. The expert group therefore recommends to support, from a molecular point of view, the functional origin of the clinical and epidemiologic observations of dioxin effects and the interspecies variability as well as the interindividual variability of these effects in humans, in particular that associated with genetic polymorphisms. This research should make it possible to end up with the best match between the mechanisms demonstrated and the choice of models for the risk assessment.

The mechanism by which 2,3,7,8-TCDD induces tumors has not been elucidated : in particular, we do not know today if this molecule is a complete carcinogen. Although not mutagenic, 2,3,7,8-TCDD may indirectly lead (*via* cytochrome induction) to oxidative DNA lesions, provoke the inhibition of apoptosis, and induce proliferation by its cytotoxic effects. The expert group recommends a study of the tumor-promoting activity of 2,3,7,8-TCDD after genotoxic lesions induced by other routes.