5. Summary of Data Reported and Evaluation

5.1 Exposure data

Polychlorinated dibenzo-*para*-dioxins (PCDDs) are formed as inadvertent by-products, sometimes in combination with polychlorinated dibenzofurans (PCDFs), during the production of chlorophenols and chlorophenoxy herbicides, and have been detected as contaminants in these products. PCDDs and PCDFs also may be produced in thermal processes such as incineration and metal-processing and in the bleaching of paper pulp with free chlorine. The relative amounts of PCDD and PCDF congeners produced depend on the production or incineration process and vary widely.

PCDDs are ubiquitous in soil, sediments and air. Excluding occupational or accidental exposures, most human exposure to PCDDs occurs as a result of eating meat, milk, eggs, fish and related products, as PCDDs are persistent in the environment and accumulate in animal fat. Occupational exposures to PCDDs at higher levels have occurred since the 1940s as a result of production and use of chlorophenols and chlorophenoxy herbicides. Even higher exposures have occurred sporadically in relation to accidents in these industries.

Mean background levels of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (2,3,7,8-TCDD) in human tissues today are in the range of 2–3 ng/kg fat. Available data suggest that these levels have decreased by a factor of 3 to 5 since the late 1970s, when the development of gas chromatography/mass spectrometry methodology first permitted these extremely low levels of PCDDs in tissues and the environment to be measured accurately. Similarly, since the mid-1980s, mean tissue levels of total PCDDs and PCDFs (measured as inter-

national toxic equivalents (I-TEQs)) in the general population have decreased by two- to three-fold. Human exposures related to occupation or accidents have led to tissue levels of 2,3,7,8-TCDD up to several orders of magnitude higher than background levels.

5.2 Human carcinogenicity data

In the evaluation of the evidence of carcinogenicity of 2,3,7,8-TCDD, more weight has been given to studies with direct 2,3,7,8-TCDD measurements and to studies involving heavy exposure to herbicides likely to be contaminated with 2,3,7,8-TCDD. The effects of 2,3,7,8-TCDD and those of the products in which it was found cannot be separated in most of the epidemiological studies; however, the focus here is on the contaminant.

The most important studies for the evaluation of the carcinogenicity of 2,3,7,8-TCDD are four cohort studies of herbicide producers (one each in the United States and the Netherlands, two in Germany), and one cohort of residents in a contaminated area from Seveso, Italy. These studies involve the highest exposures to 2,3,7,8-TCDD among all epidemiological studies, although the exposures at Seveso were lower and the follow-up shorter than those in the industrial settings. In addition, the multi-country cohort study from IARC is of special interest because it includes three of four high-exposure cohorts and other industrial cohorts, many of them not reported in separate publications, as well as some professional applicators. Most of the four industrial cohorts include analyses of sub-cohorts considered to have the highest exposure and/or longest latency. These cohorts, and their respective high-exposure sub-cohorts, are the focus of the summary here. Additional studies of herbicide applicators, both cohort and case–control studies, who have considerably lower exposures to 2,3,7,8-TCDD, are not considered critical for the evaluation.

An increased risk for all cancers combined is seen in the cohort studies cited above. The magnitude of the increase is generally low; it is higher in sub-cohorts considered to have the heaviest 2,3,7,8-TCDD exposure within the cohorts listed above. Furthermore, statistically significant positive dose–response trends for all cancers combined were present in the largest and most heavily exposed German cohort. A positive trend ($p = 0.05$) was also seen in the smaller German cohort where an accident occurred with release of large amounts of 2,3,7,8-TCDD; the positive trend in this cohort was limited to smokers. Cumulative dose in both these trend analyses was estimated by combining data from blood 2,3,7,8-TCDD levels and knowledge of job categories, work processes and calendar time of exposure. Increased risks for all cancers combined were also seen in the longer-duration longer-latency sub-cohort of the United States study. These positive trends with increased exposure tend to reinforce the overall positive association between all cancers combined and exposure, making it less likely that the increase is explained by confounding, either by smoking or by other carcinogenic exposures in the industrial setting.

An increased risk for lung cancer is also present in the most informative cohort studies, again especially in the more highly exposed sub-cohorts. The relative risk for lung cancer in the combined highly exposed sub-cohorts was estimated to be 1.4 (statis-

tically significant). It is possible that lung cancer relative risks of this order could result from confounding by smoking, but only if there were a pronounced difference in smoking habits between the exposed population and the referent populations, a difference which seems unlikely. It therefore seems unlikely that confounding by smoking can explain all the excess lung cancer risk, although it could explain part of it. It is also possible that other occupational carcinogens, many of which would affect the lung, are causing some confounding.

An excess risk for soft-tissue sarcoma, based on a small number of deaths, has been reported. Incidence data for soft-tissue sarcoma were generally not available. A case–control study nested in the IARC international cohort found a dose–response relationship with estimated 2,3,7,8-TCDD exposure; however, strong positive trends were also found with estimated exposure to 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T). A similar increase in soft-tissue sarcoma was present in the Seveso population, but only in the zone which overall had the lowest exposure. No such increase is present in the German or Dutch cohort studies. Soft-tissue sarcomas are subject to serious misclassification on death certificates; although it is unlikely that this occurs differentially in the exposed and the referent populations, reclassification of a few cases would have important consequences on results based on small numbers.

An increased risk for non-Hodgkin lymphoma was found in most of the populations studied in the four industrial cohort studies and in the Seveso population, although the relative risks were mostly nonsignificant and below 2. A case–control study nested in the IARC international cohort provided weak evidence of a dose–response relationship with estimated 2,3,7,8-TCDD exposure. Although it is plausible that other chemicals cause non-Hodgkin lymphoma, strong potential confounding factors are not known. The lack of complete consistency among the studies and the weak effect detected in most of the positive ones, however, caution against a causal interpretation of the findings.

Increased risks for several other malignant neoplasms have been sporadically reported among workers exposed to 2,3,7,8-TCDD, and at Seveso, perhaps most notable being for digestive system cancers and multiple myeloma. The available results are not fully consistent, and several studies have not reported the results for each individual cancer site.

Overall, the strongest evidence for the carcinogenicity of 2,3,7,8-TCDD is for all cancers combined, rather than for any specific site. The relative risk for all cancers combined in the most highly exposed and longer-latency sub-cohorts is 1.4. While this relative risk does not appear likely to be explained by confounding, this possibility cannot be excluded. There are few examples of agents which cause an increase in cancers at many sites; examples are smoking and ionizing radiation in the atomic bombing survivors (for which, however, there are clearly elevated risks for certain specific cancer sites). This lack of precedent for a multi-site carcinogen without particular sites predominating means that the epidemiological findings must be treated with caution; on the other hand, the lack of precedent cannot preclude the possiblity that in fact 2,3,7,8-TCDD, at high doses, does act as a multi-site carcinogen. It should be borne in mind that

the general population is exposed to levels far lower than those experienced by the industrial populations.

5.3 Animal carcinogenicity data

2,3,7,8-TCDD was tested for carcinogenicity by oral administration in three experiments in mice and in three experiments in rats. It was also tested by exposure of immature mice and by intraperitoneal or subcutaneous injection in one study in hamsters, and by skin application in mice.

In three experiments in two strains of mice, administration of 2,3,7,8-TCDD orally by gastric instillation increased the incidence of hepatocellular adenomas and carcinomas in both males and females. In one of these three experiments, 2,3,7,8-TCDD increased the incidence of follicular-cell adenomas of the thyroid, lymphomas and subcutaneous fibrosarcomas in female mice; a trend for an increased incidence of alveolar/bronchiolar adenomas or carcinomas in male mice was also observed.

Oral administration of 2,3,7,8-TCDD by gastric instillation or in the diet to rats increased the incidence of benign hepatocellular neoplasms (identified as adenomas, neoplastic nodules and hyperplastic nodules) in females in two strains and the incidence of hepatocellular carcinomas in one strain. An increased incidence of follicular-cell adenomas of the thyroid in male and female rats in the study with administration by gastric instillation was reported. In the feeding study, 2,3,7,8-TCDD increased the incidence of squamous-cell carcinomas of the tongue, hard palate, nasal turbinates and lung in both sexes of rats. In the feeding study, a high incidence of endocrine-related tumours (pituitary adenomas, phaeochromocytomas and pancreatic islet-cell tumours) was observed in control female rats. The incidence of these tumours was lower after treatment with 2,3,7,8-TCDD, associated with decreased body weight.

In one experiment involving oral administration to immature mice of two strains, 2,3,7,8-TCDD increased the incidence of hepatocellular adenomas and carcinomas in males and that of hepatocellular adenomas in females of one strain. Treatment of immature mice increased the incidence of thymic lymphomas in male and female mice of both strains.

Application of 2,3,7,8-TCDD to the skin increased the incidence of dermal fibrosarcomas in female mice. Intraperitoneal or subcutaneous administration of 2,3,7,8-TCDD to small groups of hamsters increased the incidence of squamous-cell carcinomas of the skin.

In several studies in mice, administration of 2,3,7,8-TCDD following administration with known carcinogens enhanced the incidences of skin papillomas, lung adenomas, liver adenomas and hepatoblastomas. 2,3,7,8-TCDD enhanced the incidence of focal hepatic lesions in several strains of female rats following administration of various *N*-nitrosamines. In one study, 2,3,7,8-TCDD enhanced the incidence of lung carcinomas in ovariectomized compared with intact female rats following administration of *N*-nitrosodiethylamine.

In summary, 2,3,7,8-TCDD administered at low doses by different routes to rats and mice causes tumours at multiple sites. It also causes tumours in hamsters.

Dibenzo-*para*-dioxin was tested for carcinogenicity by oral administration in one experiment in mice and in one experiment in rats. No increased incidence of tumours at any site was observed in mice or rats of either sex.

2,7-Dichlorodibenzo-*para*-dioxin (2,7-DCDD) was tested for carcinogenicity by oral administration in one experiment in mice and in one experiment in rats. No increased incidence of tumours was seen at any site in rats of either sex. In male but not in female mice, an increased incidence of hepatocellular adenomas was observed, but the impurity of the chemical confounds an evaluation of its carcinogenicity. In one study, 2,7-DCDD did not enhance the incidence of skin papillomas in mice treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

A mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-*para*-dioxins was tested for carcinogenicity by oral administration in mice and in rats, and by administration to the skin in mice. The incidence of hepatocellular adenomas was increased in male and female mice and in female rats following oral administration. Impurities in the mixture were unlikely to have been responsible for the observed response. No significant increase in tumours at any site was observed following application to the skin in mice.

In other studies, administration of either 1,2,3,7,8-pentachlorodibenzo-*para*-dioxin or 1,2,3,4,6,7,8-heptachlorodibenzo-*para*-dioxin led to an increased incidence of hepatic focal lesions in female rats following treatment with nitrosamines.

Administration of a defined mixture of 49 PCDDs increased the incidence of hepatic focal lesions in female rats following treatment with *N*-nitrosomorpholine.

5.4 Other relevant data

5.4.1 *Kinetics*

In most vertebrate species, the 2,3,7,8-substituted PCDDs are the congeners which are predominantly retained. If chlorine atoms are present on all 2,3,7,8 positions, the biotransformation rate of PCDDs is strongly reduced, resulting in significant bioaccumulation. In most species the liver and adipose tissue are the major storage sites.

As Ah receptor-mediated effects are caused primarily by the parent compound, biotransformation to more polar metabolites should be considered to be a detoxification process. Although kinetics influence the biological and toxic effects, genetic factors seem to play a dominant role.

5.4.2 *Toxic effects*

Human exposure to 2,3,7,8-TCDD or other PCDD congeners due to industrial or accidental exposure has been associated with chloracne and alterations in liver enzyme levels in both children and adults. Changes in the immune system and glucose metabolism have also been observed in adults. Infants exposed to PCDDs and PCDFs through breast milk exhibit alterations in thyroid hormone levels and possible neurobehavioural and neurological deficits.

The extraordinary potency of 2,3,7,8-TCDD and related 2,3,7,8-substituted PCDDs has been demonstrated in many animal species. The lethal dose of 2,3,7,8-TCDD, however, varies more than 5000-fold between the guinea-pig, the most sensitive, and the hamster, the least sensitive species. In all mammalian species tested so far, lethal doses of 2,3,7,8-TCDD result in delayed death preceded by excessive body weight loss ('wasting').

Other signs of 2,3,7,8-TCDD intoxication include thymic atrophy, hypertrophy/-hyperplasia of hepatic, gastrointestinal, urogenital and cutaneous epithelia, atrophy of the gonads, subcutaneous oedema and systemic haemorrhage.

In tissue culture, 2,3,7,8-TCDD affects growth and differentiation of keratinocytes, hepatocytes and cells derived from other target organs. Toxicity of 2,3,7,8-TCDD segregates with the Ah receptor, and relative toxicity of other PCDD congeners is associated with their ability to bind to this receptor.

PCDDs cause suppression of both cell-mediated and humoral immunity in several species at low doses.

PCDDs have the potential to suppress resistance to bacterial, viral and parasitic challenges in mice.

5.4.3 *Effects on reproduction*

Most studies on reproductive effects of PCDDs in humans concerned paternal exposure, usually long after high exposure had occurred. Most studies have a limited power to detect elevations in specific birth defects. The studies also showed discordant results concerning an increase in the risk of spontaneous abortions. Some studies have shown alterations in hormone levels and sperm characteristics after PCDD exposure.

2,3,7,8-TCDD is both a developmental and reproductive toxicant in experimental animals. The developing embryo/fetus appears to display enhanced sensitivity to the adverse effects of PCDDs. Perturbations of the reproductive system in adult animals require overtly toxic doses. In contrast, effects on the developing organism occur at doses > 100 times lower that those required in the mother. Sensitive targets include the developing reproductive, nervous and immune systems. Perturbation of multiple hormonal systems and their metabolism due to PCDD exposure may play a role in these events.

5.4.4 *Genetic effects*

In human studies after in-vivo exposure, there have been no unequivocal reports of effects of 2,3,7,8-TCDD or other PCDD congeners upon the frequencies of chromosomal aberrations.

In animal studies *in vivo* and in cultured human and animal cells *in vitro*, 2,3,7,8-TCDD gave conflicting results with regard to several genetic endpoints, such as DNA damage, gene mutations, sister chromatid exchange and cell transformation.

Experimental data indicate that 2,3,7,8-TCDD and probably other PCDDs and PCDFs are not direct-acting genotoxic agents.

5.4.5 *Mechanistic considerations*

The administration of 2,3,7,8-TCDD in rodent bioassays significantly increased the incidence of benign and/or malignant tumours in various tissues (liver, lung, lymphatic system, soft tissue, nasal turbinates, hard palate, thyroid and tongue) in both sexes. The number of tumours per animal (multiplicity) was small. Prior exposure to a known carcinogen and subsequent exposure to 2,3,7,8-TCDD enhanced (promoted) tumour incidence and/or multiplicity and resulted in the appearance of tumours at earlier times. While 2,3,7,8-TCDD has been demonstrated to increase tumour incidence at different sites, the pattern of tumour sites is a function of species, sex and study.

2,3,7,8-TCDD is not directly genotoxic. A number of hypotheses addressing the mechanisms of 2,3,7,8-TCDD-mediated tumour promotion have been presented. These hypotheses include Ah receptor-mediated alteration in expression of networks of genes involved in cell growth and differentiation, DNA damage mediated by cytochrome P450-catalysed metabolic activation pathways, expansion of preneoplastic cell populations via inhibition of apoptosis, positive modulation of intra- or extracellular growth stimuli, or suppression of immune surveillance. For thyroid tumour induction, an indirect mechanism of 2,3,7,8-TCDD-induced carcinogenesis has also been proposed. In rodents, the induction of hepatic uridine diphosphate-glucuronosyl transferase resulted in enhanced elimination of thyroid hormones as glucuronides from the circulation, and subsequent enhanced stimulation of the thyroid gland via elevated levels of circulating thyroid-stimulating hormone.

(*a*) *Ah receptor*

The Ah receptor is a ubiquitous transcription factor found in both rodents and humans. PCDDs bind to human and rodent Ah receptors with very similar structure–activity relationships; 2,3,7,8-TCDD has the highest affinity of the PCDDs for both rodent and human receptors.

Both in humans and in mice, two forms of Ah receptor have been identified which exhibit a 5–10-fold difference in binding affinity for 2,3,7,8-TCDD. In humans, one form of the Ah receptor exhibits a K_d (a measure of binding affinity) for 2,3,7,8-TCDD of 0.4 nM, whereas the other form binds 2,3,7,8-TCDD with a K_d of about 2 nM.

In congenic mouse strains, expression of the lower or higher affinity forms of receptor has been extensively demonstrated to result in proportional differences in sensitivity to 2,3,7,8-TCDD with regard to biochemical changes and toxic effects. Thus, congenic mice expressing the lower-affinity form of receptor require higher doses of 2,3,7,8-TCDD to elicit these effects than strains expressing higher-affinity forms. A similar difference in sensitivity to PCDDs has also been demonstrated in tumour promotion studies in skin of congenic mouse strains. In these studies, PCDDs show the same rank order of potency in Ah receptor binding *in vitro* and tumour induction *in vivo*. Taken together, these data strongly support a receptor-mediated mechanism of mouse skin carcinogenesis.

(*b*) *Gene expression*

The best studied 2,3,7,8-TCDD-dependent gene expression response is the induction of *CYP1A1* and *CYP1A2*. In both rodent and human cells, this response is mediated by the Ah receptor. In rodent and human cells, PCDDs show very similar potencies in inducing *CYP1A1* and *CYP1A2* expression in rodent and human cells. The role, if any, of the induction of these genes in carcinogenesis by 2,3,7,8-TCDD is unclear. 2,3,7,8-TCDD-induced gene regulatory responses and biochemical effects documented in rodent tissues and/or cells have also been observed in human tissues or cells.

(*c*) *Comparison of tissue concentrations in humans and animals*

Four epidemiological studies of high-exposure industrial cohorts in Germany, the Netherlands and the United States found an increase in overall cancer mortality.

In these cohorts, the blood lipid 2,3,7,8-TCDD levels estimated to the last time of exposure were 2000 ng/kg (mean) (up to 32 000 ng/kg) in the United States cohort, 1434 ng/kg geometric mean (range, 301–3683 ng/kg) among accident workers in the Dutch cohort, 1008 ng/kg geometric mean in the group of workers with severe chloracne in the BASF accident cohort in Germany and measurements up to 2252 ng/kg in the Boehringer cohort in Germany. These calculated blood 2,3,7,8-TCDD levels in workers at time of exposure were in the same range as the estimated blood levels in a two-year rat carcinogenicity study. In rats exposed to 100 ng/kg bw 2,3,7,8-TCDD per day, hepatocellular carcinomas and squamous-cell carcinomas of the lung were observed. Estimated blood levels were 5000–10 000 ng/kg 2,3,7,8-TCDD. In the same study, in rats exposed to 10 ng/kg bw 2,3,7,8-TCDD per day, hepatocellular nodules and focal alveolar hyperplasia were observed. Estimated blood levels were 1500–2000 ng/kg 2,3,7,8-TCDD. These results indicate parallel tumorigenic responses to high exposure to 2,3,7,8-TCDD in both humans and rats.

In view of the results mentioned above, it should be noted that the present background levels of 2,3,7,8-TCDD in human populations (2–3 ng/kg) are 100 to 1000 times lower than those observed in this rat carcinogenicity study. Evaluation of the relationship between the magnitude of the exposure in experimental systems and the magnitude of the response (i.e., dose–response relationships) do not permit conclusions to be drawn on the human health risks from background exposures to 2,3,7,8-TCDD.

5.5 Evaluation[1]

There is *limited evidence* in humans for the carcinogenicity of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin.

There is *sufficient evidence* in experimental animals for the carcinogenicity of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin.

There is *evidence suggesting lack of carcinogenicity* in experimental animals for dibenzo-*para*-dioxin.

[1] For definition of the italicized terms, see Preamble, pp. 26–27.

There is *limited evidence* in experimental animals for the carcinogenicity of a mixture of 1,2,3,6,7,8- and 1,2,3,7,8,9-hexachlorodibenzo-*para*-dioxins.

There is *inadequate evidence* in experimental animals for the carcinogenicity of 2,7-dichlorodibenzo-*para*-dioxin.

There is *inadequate evidence* in experimental animals for the carcinogenicity of 1,2,3,7,8-pentachlorodibenzo-*para*-dioxin.

There is *inadequate evidence* in experimental animals for the carcinogenicity of 1,2,3,4,6,7,8-heptachlorodibenzo-*para*-dioxin.

Overall evaluation

2,3,7,8-Tetrachlorodibenzo-*para*-dioxin is *carcinogenic to humans (Group 1).*

In making the overall evaluation, the Working Group took into consideration the following supporting evidence:

(i) 2,3,7,8-TCDD is a multi-site carcinogen in experimental animals that has been shown by several lines of evidence to act through a mechanism involving the Ah receptor;

(ii) this receptor is highly conserved in an evolutionary sense and functions the same way in humans as in experimental animals;

(iii) tissue concentrations are similar both in heavily exposed human populations in which an increased overall cancer risk was observed and in rats exposed to carcinogenic dosage regimens in bioassays.

Other polychlorinated dibenzo-*para*-dioxins are *not classifiable as to their carcinogenicity to humans (Group 3).*

Dibenzo-*para*-dioxin is *not classifiable as to its carcinogenicity to humans (Group 3).*