Dioxins

Toxicological Overview

Key Points

Identity

- Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo-p-furans ("dioxins") is a term used to refer to a group of 210 compounds with similar chemical structures
- Most of these compounds pose no health hazard at the levels commonly found but 17 of them are of more toxicological concern
- The most toxic dioxin is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and most of the available data refer to this compound

Kinetics and metabolism

- Dioxins are readily absorbed following ingestion and are also likely to be absorbed following inhalation or dermal exposure
- Once absorbed they are extensively distributed throughout the body, with particular accumulation in the liver and adipose tissue
- The metabolism of dioxins is extremely slow, with partial excretion in the faeces as metabolites. They are extremely persistent with the elimination half-life of TCDD being 7 to 12 years, therefore there is potential for accumulation in body tissues

Health effects of acute exposure

- The characteristic adverse effect following a severe acute exposure to dioxins is chloracne, the onset of which may be delayed several months
- Acute exposure to dioxins may also cause nausea, vomiting, diarrhoea, hepatic damage and neurological effects

Health effects of chronic exposure

- The adverse effects of chronic exposure to dioxins are similar to those following acute exposure
- Chronic exposure to dioxins may also cause liver disease, increased risk of developing diabetes, alterations in thyroid function, impaired immune function, cardiovascular disease, mild neuropathies and developmental effects
- TCDD is classified by IARC as being carcinogenic to humans

Toxicological Overview

Summary of Health Effects

Dioxin is a term used to describe a group of closely related compounds with similar chemical structures but which vary greatly in their toxicity. These include 75 polychlorinated dibenzo-p-dioxins and 135 polychlorinated dibenzo-p-furans, the most toxic being 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). TCDD is the most extensively studied of the dioxins and most of the data in this document refer to TCDD.

Dioxins are well absorbed following oral exposure and undergo extensive distribution throughout body tissues, with particular accumulation in the liver and adipose tissues [1]. Dioxins are also expected to be well absorbed following inhalation and dermal exposure. Dioxins are not rapidly eliminated and are extremely persistent, the half-life of TCDD in humans is considered to approximately 7 to 12 years [2]. Due to the extensive distribution and persistence of absorbed dioxins there is a high potential for accumulation following repeated exposure.

The most common adverse health effect observed in humans following acute exposure to dioxins, particularly TCDD, is the development of chloracne. Chloracne is an acne-like condition characterised by follicular hyperkeratosis which may include cysts and pustules and typically involves the hair follicles on the face and neck [1-3]. The onset of chloracne in individuals following acute exposure to dioxins may develop within months of exposure to relatively high levels. The delay in onset of chloracne is, however, dependent upon the duration and concentration of exposure. Mild cases of chloracne may clear several months post-exposure, however, in severe cases the lesions may be present 30 years after the initial onset [2]. Acute exposure to high levels of dioxins (particularly TCDD) may also cause nausea, vomiting and diarrhoea, hepatic damage and neurological effects, including headaches, weakness, muscular pains, peripheral neuropathy, loss of appetite, sexual dysfunction and transient weight loss [1, 2].

The adverse effects in humans of chronic or repeated exposure to dioxins are similar to those observed following severe acute exposure. Chloracne and alterations in hepatic enzyme levels have been seen in individuals occupationally exposed to dioxins [2, 3]. Other effects which have been reported include increased mortality from non-malignant liver disease, increased risk of developing diabetes, alterations in thyroid function, impaired immune function, increased mortality from cardiovascular disease, mild neuropathies and neurobehavioural effects in children [3].

TCDD is a reproductive and developmental toxin. There have been some reports to suggest that exposure to low levels of dioxins in the environment may cause subtle delays in development and alterations in thyroid function to the children of mothers exposed during pregnancy [3]. The most sensitive effect seen in toxicity studies in animals was developmental toxicity, the effects seen including induction of abortion, changes in behavioural and cognitive function and adverse effects on the developing male reproductive system [4]. The latter was the basis for the recommended Tolerable Daily Intake (TDI) [5].

The International Agency for Research on Cancer (IARC) has classified TCDD as being carcinogenic to humans (Group 1). However, other polychlorinated dibenzo-*p*-dioxins are not classifiable as to their carcinogenicity in humans (Group 3) [4]. This was based on limited evidence of induction of cancer from epidemiology studies in occupationally exposed

workers, plus evidence that TCDD was a multi-site animal carcinogen and that the mechanism of its toxicity was similar in humans and animals [4]. The weight of evidence from the available experimental studies indicates that dioxin is not a genotoxic agent [3].

Introduction

Dioxins is a term used to describe a group of 210 closely related compounds with similar chemical structures but varying greatly in their toxicity. They comprise 75 polychlorinated dibenzo-p-dioxins and 135 polychlorinated dibenzofurans. TCDD is the most toxic of the dioxins and is the most extensively studied. Most of the data in this document refer to TCDD.

Exposure to dioxins is most likely to be in the form of a mixture of related dioxin compounds with differing potency. Therefore, a system has been devised to consider the toxicity of a given mixture. The system is referred to as the 'toxic equivalent approach' and is based on weighting the individual compounds present in the mixture compared to the most potent compound (TCDD). This approach is recommended by the World Health Organisation (WHO) and has been endorsed by the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) [2, 3].

Kinetics and Metabolism

Studies of the kinetics of dioxins following oral exposure have demonstrated that they are well absorbed and undergo extensive distribution throughout body tissues, with particular accumulation in the liver and adipose tissues [1]. A human volunteer study estimated the absorption of TCDD to be greater than 87% [2]. Dioxins are not rapidly eliminated and are extremely persistent, the half-life of TCDD in humans being approximately 7 to 12 years [2]. Due to the extensive distribution and persistence of absorbed dioxins there is a high potential for accumulation following repeated exposure. No data were available regarding the uptake of dioxins by inhalation in humans, however, data from animal studies suggest that dioxins are well absorbed following inhalation exposure [2]. No data were available on the absorption of dioxins following dermal exposure, but studies with structurally related chemicals suggest that dermal absorption is possible.

Relatively little is known about the metabolism of dioxins in humans, however, there is some evidence to suggest that absorbed TCDD may be slowly converted to polar metabolites that are eliminated as glucuronates [5]. Studies in animals suggest that the metabolism of absorbed dioxins is very slow, giving rise to its long elimination half-life [1, 2].

Sources and Route of Human Exposure

Dioxins are not deliberately manufactured. Low level exposure can occur from the presence of dioxins in combustion processes such as incineration of municipal waste, domestic fires and bonfires and internal combustion in automobile engines; as impurities in certain manufactured chemicals or during some industrial processes.

The greatest potential for occupational exposure to dioxins is to workers involved in the production, use and decomposition of materials which may contain small amounts of dioxins as impurities or from combustion processes in which dioxins may be formed. The major routes of occupational exposure to dioxins are by inhalation and by dermal contact [1, 2].

The most common source of non-occupational exposure to dioxins is from the ingestion of food products such as meat, dairy products and fish containing low levels of dioxins. Slightly higher levels may be found in foods from areas where pollution from certain organochlorine chemicals contaminated with dioxins may have occurred in the past [2].

The majority of studies on the effects of human exposure to dioxins have come from cases of accidental, occupational and residential exposure. In many of these cases there is little or no accurate exposure data, with no monitoring of exposure levels or internal doses [2].

Health Effects of Acute / Single Exposure

Human Data

General toxicity

The most common adverse health effect observed in humans following acute exposure to dioxins, particularly TCDD, is the development of chloracne. Chloracne is an acne-like condition characterised by follicular hyperkeratosis which may include cysts and pustules. Chloracne typically involves the hair follicles on the face and neck, but may also include the upper arms, back, chest, abdomen, outer thighs and genitalia [1-3]. Mild cases of chloracne may clear several months post-exposure; however, in severe cases the lesions may still be present 30 years after the initial onset [2].

Acute exposure to relatively high levels of dioxins (particularly TCDD) may give rise to effects including nausea, vomiting, diarrhoea and transient weight loss. Neurological effects have also been commonly observed including headaches, weakness, muscular pains and peripheral neuropathy [1, 2]. Acute exposure to dioxins has also been associated with hepatic damage observed by alterations in enzyme levels such as increases in transaminase levels in the blood, hypercholesteraemia and hypertriglyceridaemia [1, 2, 4]. Sexual dysfunction has also been reported [1, 2].

The available case studies concerning human exposure to dioxins do not give accurate data regarding route or exposure concentration. However, the adverse health effects resulting from acute exposure to dioxins are expected to be similar whether by inhalation, ingestion or dermal exposure [2].

Delayed effects following an acute exposure

The onset of chloracne in individuals following acute exposure to relatively high levels of dioxins may develop within months of exposure, the delay in onset being dependent upon the duration and concentration of exposure. The delayed onset in adults has been shown to develop initially as irritative lesions within 20 to 40 days, which later develop into chloracne. The development of chloracne in an adult population in Seveso, Italy following an accidental release of TCDD was shown to range from 3 to 10 months post-exposure and its severity was shown to progressively decrease in the 2 years post-exposure. In children exposed to TCDD, the onset of chloracne was observed within 2 weeks and persisted between 8 and 26 months [2].

Animal and In-Vitro Data

Ingestion

The oral toxicity of TCDD varies widely between species from an oral LD_{50} of just 0.6 µg kg^{-1} body weight (bw) in male Hartley guinea pigs, to relatively lower toxicity in the Syrian hamster with an oral LD_{50} of 5051 µg kg^{-1} bw. The oral LD_{50} for TCDD in male Sprague Dawley rats is 43 µg kg^{-1} bw. Death is delayed, occurring 2-3 weeks post exposure [2, 3].

A characteristic effect of TCDD toxicity in experimental animals following a single oral exposure is a significant reduction in body weight known as wasting syndrome and is the most common cause of death in experimental animals [2]. The wasting syndrome is characterised by inhibition of gluconeogenesis, reduced feed intake and loss of body weight [6].

The liver is considered to be a principal target of toxicity for dioxins as adverse effects upon the liver have been observed in many experimental animal studies, including rats and mice following an acute oral exposure to TCDD [2]. Biochemical changes including hypoglycaemia and increased serum triglyceride and cholesterol levels were observed in Fisher 344 rats 10 days post-exposure to a single sub-lethal oral dose of TCDD, indicating hepatic damage. Histological changes including enlarged hepatocytes with centrilobular vacuolated cytoplasm and cellular degeneration in the centrilobular region have also been observed in rats following a single acute exposure to TCDD [2]. TCDD has also been shown to cause hepatomegaly in all species tested resulting from hypertrophy and hyperplasia of parenchymal cells [1].

Acute exposure of rhesus monkeys to a single oral dose of TCDD of 70 µg kg^{-1} has been shown to induce dermal lesions resembling the chloracne observed in humans. The dermal effects included oedema and inflammation of the eyelids, loss of nails and loss of facial hair with the presence of acne-like lesions [1, 2].

Additional effects observed in animals administered a single acute oral dose of TCDD include atrophy of the thymus and also the spleen and lymph nodes [1, 2].

Inhalation

No studies were available regarding the acute toxicity of dioxins in experimental animals following inhalation exposure.

Dermal / ocular exposure

The information regarding acute dermal exposure of dioxins to experimental animals is limited. However, the dermal LD$_{50}$ for TCDD in rabbits has been calculated at 275 µg kg^{-1}, with death occurring 12-22 days post-exposure [2].

In the limited dermal exposure studies of TCDD, similar effects have been observed as those seen following oral exposure, including hepatic effects and decreased body weight. Dermal effects, including hyperkeratosis and epidermal hyperplasia, have been observed in hairless mice following dermal exposure to 0.1 µg kg^{-1} TCDD [2].

Health Effects of Chronic / Repeated Exposure

Human Data

General toxicity

The adverse effects in humans following chronic or repeated exposure to dioxins are similar to those observed following severe acute exposure. Chloracne and alterations in hepatic enzyme levels have been seen in individuals occupationally exposed to dioxins. Positive correlations have been noted between serum and adipose levels of TCDD and increased risk of chloracne, hyperpigmentation and abnormal distribution of hair in occupationally exposed chemical production workers [2, 3]. Other effects that have been reported include increased mortality from non-malignant liver disease, increased risk of developing diabetes, alterations in thyroid function, impaired immune function, increased mortality from cardiovascular disease, mild neuropathies and neurobehavioural effects in children [3].

It is not possible to determine the concentration of TCDD or duration of exposure which gives rise to these adverse effects following chronic exposure since in most cases the data are limited [2].

In many cases of repeated exposure to TCDD resulting in the formation of chloracne, removal from the exposure will eventually result in the disappearance of the condition. However, due to the extremely long elimination half-life of TCDD, the condition may remain for 25 years or more [2].

Genotoxicity

Studies investigating chronic exposure of humans to either TCDD or other dioxin congeners have not reported any evidence of genotoxic effects [4]. No significant differences were reported in the incidence of chromosomal aberrations or sister chromatid exchange in exposed individuals, although there were limitations in the exposure data [2].

Carcinogenicity

The IARC summarised data from populations with the highest exposures to TCDD and suggested that there is strong evidence for increased risk of cancer at all sites combined, and some evidence for increased risk of specific cancers such as non-Hodgkins lymphoma, multiple myeloma or digestive system cancers, but commented that there was little consistency between studies [3, 4].

IARC concluded that there is limited evidence in humans for the carcinogenicity of TCDD but overall, classified it as being carcinogenic to humans (Group 1) [4]. When making this classification, the working group took into account the fact that TCDD is a multi-site carcinogen in experimental animals and that the mechanism involves the aromatic hydrocarbon (Ah) receptor protein, which regulates some specific gene expression associated with toxicity. Binding to this receptor is an integral part of the toxicological mechanism of a range of chemicals including dioxins. The Ah receptor is highly conserved and functions in the same way in humans as animals. In addition, the tissue concentrations were similar in the heavily exposed human populations showing increased cancer and the rat bioassay data [4].

Other polychlorinated dibenzo-*p*-dioxins are not classifiable as to their carcinogenicity in humans (Group 3) [4].

Reproductive and developmental toxicity

A number of studies have investigated the association between chronic exposure to TCDD and reproductive toxicity in humans. However, many of the studies lack adequate exposure data [2].

Most studies investigating the reproductive effects of exposure to dioxins in humans have involved paternal exposure to high doses although most are limited in their ability to detect any increases in specific birth defects. Exposure to dioxins has been suggested in some studies to cause alterations in hormone levels and sperm characteristics and, in some cases, an increase in the risk of spontaneous abortions was noted. In contrast, in other studies the evidence for this effect was not conclusive [4]. Data arising from the population exposed to dioxin following the Seveso industrial accident in 1976 indicated that the sex ratio of children born to parents living in the zone with the highest level of contamination was altered in favour of females. Such effects were seen in the births occurring from 9 months after the incident to 7 years later, with about twice as many children being female [6].

There have been some reports to suggest that exposure to low levels of dioxins in the environment may cause subtle delays in development and alterations in thyroid function to the children of mothers exposed during pregnancy. However, due to limitations of the data it was not possible to determine whether the effects were associated solely with dioxin exposure. However, a recent study on mothers exposed to the Seveso accident appears to confirm long last effects on the thyroid in their children [3].

Animal and In-Vitro Data

Ingestion

The adverse effects in animals following chronic exposure to dioxins are similar to those observed following severe acute toxicity. Chronic dietary exposure of Sprague-Dawley rats to 0.1 µg kg^{-1} day^{-1} TCDD has been shown to give rise to a significant reduction in body weight (wasting syndrome), and increased mortality was reported in Swiss and B6C3F1 mice given 0.36 or 1 µg kg^{-1} day^{-1} TCDD by gavage once a week for a year [2].

Additional effects resulting from repeated exposure to dioxins include reduced food intake, atrophy of the thymus, hypertrophy and hyperplasia of the hepatic, gastrointestinal and cutaneous epithelia, liver necrosis and gastrointestinal haemorrhage [2-4].

Inhalation

No studies were available on the chronic toxicity of dioxins in experimental animals following inhalation exposure [2].

Genotoxicity

The potential of TCDD to induce genetic mutation has been extensively studied in the Ames test *in vitro* using strains of *Salmonella typhimurium* and has been shown to be predominantly negative in these assays [2]. TCDD has also been investigated for genotoxic potential in *in-vitro* systems using cultured mammalian cells. No evidence of unscheduled DNA synthesis (an indirect marker of DNA damage) was observed in cultured human cells. However, gene mutations were observed in mouse lymphoma cells and sister chromatid exchange was seen in Chinese hamster ovary cells [2]. The design of many of the studies

was limited and inconclusive. However, TCDD does not appear to have direct-acting genotoxic potential *in vitro* as results were largely negative for a range of genotoxic endpoints [2, 4].

In-vivo animal studies carried out to investigate the genotoxic potential of TCDD have been found to be predominantly negative. Cytogenetic analysis of the bone marrow did not reveal any increase in the incidence of chromosomal aberrations in CD-COBS rats orally administered TCDD, but an increase was observed in Osborne-Mendel rats. No increase in the incidence of chromosomal aberrations was reported in the peripheral lymphocytes of monkeys exposed to TCDD for 4 years [2]. Data from in-vivo experimental studies suggest that TCDD or other dioxin congeners do not have mutagenic potential [2, 4]. The COM most recently reviewed the available mutagenicity data in 1999. They concluded that the weight of available experimental evidence continued to indicate that TCDD is not a genotoxic agent [7].

Carcinogenicity

In three studies in which two strains of mouse were orally administered TCDD, an increase in the incidence of hepatocellular adenomas and carcinomas was observed in both the males and females. In one study an increase in the incidence of follicular-cell adenomas of the thyroid, lymphomas and subcutaneous fibrosarcomas was observed in the female mice, and an increase in the incidence of alveolar and bronchiolar adenomas or carcinomas was reported in male mice [4].

Low dose exposure to TCDD has been shown to cause tumours at multiple sites in rats and mice including the liver and thyroid gland [4]. In a comprehensive 2-year feeding study in the rat the top dose of 100 ng kg^{-1} day^{-1} clearly produced an increase in malignant liver tumours. The intermediate dose level, 10 ng kg^{-1} day^{-1} was considered to be the Lowest Observed Adverse Effect level (LOAEL) and the low dose level, 1 ng kg^{-1} day^{-1} was considered not to cause any adverse effects [3].

IARC has concluded that there is sufficient evidence in experimental animals for the carcinogenicity of TCDD [4]. However, it also concluded that there is evidence to suggest that there is a lack of carcinogenicity in experimental animals for dibenzo-*p*-dioxin and there is inadequate evidence for the carcinogenicity of other chlorinated dibenzo-*p*-dioxins [4].

Reproductive and developmental toxicity

Exposure to TCDD has been shown to cause reproductive and developmental toxicity in experimental animals [1-4].

Chronic administration of TCDD to rats in a three generation reproductive study caused significantly decreased fertility in the F_1 and F_2 generations, but not in the F_0 generation. Fetal anomalies, including cleft palate and kidney anomalies have been observed in the offspring of mice exposed to TCDD during pregnancy at doses below those required to cause fetotoxicity or maternal toxicity. The offspring of rats exposed to TCDD during pregnancy have displayed thymic atrophy and cell-mediated immune suppression at doses much lower than those required to cause maternal toxicity [3, 4].

Developmental toxicity is the most sensitive toxic effect of TCDD observed in animal studies. The effect on the developing reproductive system of the male rat fetus is considered to be the most sensitive indicator of TCDD toxicity [5, 6]. The COT estimated that reduced sperm count in the male offspring was occurring at TCDD body burden of 33 ng kg^{-1} in the mothers on day 16 of pregnancy [5].

References

[1]   International Programme on Chemical Safety (IPCS) (1989). Polychlorinated dibenzo-p-dioxins and dibenzofurans. Environmental Health Criteria 88. WHO. Geneva.

[2]   Agency for Toxic Substances and Disease Registry (ATSDR) (1998). Toxicological Profile for Chlorinated Dibenzo-p-Dioxins. US department of Health and Human Services. Atlanta, US.

[3]   Department for Environment Food and Rural Affairs (DEFRA) and Environment Agency (EA) (2003). Contaminants in soil: Collation of toxicological data and intake values for humans. Dioxins, Furans and Dioxin-like PCBs. Environment Agency. Bristol.

[4]   International Agency for the Research on Cancer (IARC) (1997). Polychlorinated Dibenzo-para-Dioxins and Polychlorinated Dibenzofurans. Vol 69. IARC. Lyon.

[5]   Committee on Toxicity of Chemicals in Food Consumer Products and the Environment (COC) (2001). Statement on the Tolerable Daily Intake for dioxins and dioxin-like polychlorinated biphenyls.

[6]   World Health Organisation (WHO) (2002). Safety evaluation of certain food additives and contaminants. Polychlorinated dibenzodioxins, polychlorinated dibenzofurans and coplanar polychlorinated biphenyls. WHO food additives series No 48. WHO. Geneva.

[7]   Committee on Mutagenicity of Chemicals in Food Consumer Products and the Environment (COC) (1999). Statement on TCDD. COM/99/S2.